THE ADRENAL CIRCULATION

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PREFACE

For some fifteen years R. G. H. has been interested in the functional morphology of the adrenal gland, with particular reference to its blood supply. Only as a result of researches carried out over the last few years, however, has it become apparent how the blood flow within the gland may become modified as a result of direct action upon its intracortical vessels. The mechanism of this action was first investigated in the rabbit, but in the years 1958-59, an extensive study on the vascularization of the adrenal cortex in the rat was undertaken by M. J. H. for the B.Sc. Honours degree in Anatomy in this Department, and by R. G. H. on the rat and monkey. It was considered that these researches, combined together in integrated form, would warrant publication as a monograph, particularly since, if they had been published as separate papers, their individual significance may have been obscure. We are aware that many aspects of this study could warrant further investigation, and research on these lines is still being actively pursued, but in view of the importance of these discoveries, it seemed that publication of the researches effected so far was indicated.

Our thanks are due to the technical staff of this Department for their assistance in much of the experimental work described in this book, and in particular Mrs. C. Morley for preparation of the histological material, and Mr. A. Taunton for the photography. We are also indebted to Miss M. R. Crowther for typing the manuscript, and to Mr. L. G. Cooper for photographing and Mr. D. J. Kidd for drawing some of the illustrations in this book. The researches described herein have been financially assisted by grants from the Medical Research Council, and the Sir Halley Stewart Trust. We also wish to express our gratitude to Damancy & Co. Ltd (and in particular Mr. P. G. Horlington) for providing facilities for the viscosity measurements described in Table I; Professor Barry J. Anson, Department of Anatomy, Northwestern University Medical School, Chicago, for permission to reproduce Fig 14; Dr. W. J. Tindall, Organon Laboratories Ltd. for supplying the hormones utilized in these researches; Dr. A. Spinks and Dr. J. S. Lowe of Imperial Chemical Industries Ltd. (Pharmaceuticals Division) for supplying the serotonin, ox kallidin and adrenochrome; Professor A. Wilson, Department of Pharmacology, University of Liverpool, for providing the Levophed and Dibenamine used in these experiments; and to Dr. J. L. Braithwaite, Professor A. Haddow and the Chester Beatty Research Institute, for providing the tumour-bearing rats used in the experiments described on p. 58.

Finally, we wish to thank Mr. Per Saugman, the staff of Blackwell Scientific Publications and the Alden Press for their continued help in the preparation of this book.

RONALD G. HARRISON

Liverpool, May 1960

M. JUNE HOEY

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CHAPTER I

INTRODUCTION

Claude Bernard (1858) claimed that the state of the venous blood which issues from secretory organs differs according to whether the organ is functioning or resting. Further, that this relationship between blood draining the gland and glandular activity is determined by the activity of the nervous system

For some time it has been claimed that the level of adrenal cortical secretion is governed in large part, if not entirely, by the anterior lobe of the hypophysis cerebri. Any influence of the nervous system on the physiological state of the adrenal cortex has been considered (Harris, 1955) to be effected through the intermediary of the hypothalamus, the hypophyseal portal circulation, the anterior lobe of the pituitary and thereby its secretion of adrenocorticotrophic hormone (ACTH), which in turn stimulates the secretion of steroid hormones from the cortex of the adrenal gland. Although such a complicated mechanism is called into play in certain circumstances, complete acceptance of it precludes any *direct* effect of hormones or environmental change on adrenal cortical secretion, it does not explain the increasingly close topographical association of adrenal cortex and medulla in non-aquatic vertebrates during the evolutionary process, and has laid so much stress on the importance of the activity of the anterior lobe of the pituitary, that manifestations of the action of adrenal cortical hormones are now frequently interpreted *ipso facto* as an indicator of ACTH secretion.

Many of the investigations on the regulation of the secretion of adrenal cortical steroids by the pituitary have employed adrenaline or some other vasoconstrictor. Yet recent experiments (Harrison, 1957) have demonstrated that adrenaline has a direct effect on the vascularization of the adrenal cortex of the rabbit independent of the hypothalamus and pituitary, since an increase in adrenal cortical vascularization could be obtained in the decerebrate, hypophysectomized rabbit following the injection of adrenaline.

Since 1957 many additional experiments, to be reported here for the first time, have confirmed that adrenaline, other vasoconstrictor agents and the submission of an animal to stress, may have a similar direct effect on the adrenal cortex by increasing its vascularization. It was therefore deemed advisable to collate these observations and publish them as a monograph in conjunction with a description of the gross anatomy of the adrenal circulation, in view of the knowledge of the anatomy of the adrenal gland now made necessary by the increased frequency of adrenalectomy in surgical practice.

stand out clearly as white strands and after formalin fixation they can be dissected more easily, since the Micropaque then remains in the vessels, and does not diffuse into surrounding tissues, a disadvantage possessed by other radiopaque media which are solutions, such as those commonly used in radiography in clinical practice. Since these latter solutions depend for their radiopacity on their content of iodine they are also not as radiopaque as barium sulphate. Other radiopaque media (Harrison, 1951a) have a larger particle size and do not penetrate as far into the finer branches of the vascular system. Thus, at one time bismuth oxychloride suspension was considered ideal, but it was later found that Micropaque is more effective. The Micropaque suspension as provided by the manufacturers is at a strength of 100% w/v but it is usually found more desirable to dilute this 50% with water or saline, thus providing a 50% suspension. Generally, the weaker the suspension, the more effective the filling of the finest vessels (see Brookes and Harrison, 1957), and this may be explained by the lower viscosity of the weaker suspensions (Table I). Preparations obtained by the injection of latex rubber or plastic injection masses have usually been found unsuitable and more tedious to prepare in this laboratory: generally unphysiological injection pressures have to be used in order to fill even small arteries, subsequent corrosion of the tissue is necessary, and radiography prior to dissection is either impossible or unsatisfactory. Similar criticisms may be made against the use of other injection media, such as carmine gelatine or india ink for the visualization of the gross anatomy of the circulatory system.

The gross arterial supply of the right and left adrenal glands has been examined in twenty albino rats, eight rabbits, four cats and four macaque monkeys following injection of Micropaque into the descending thoracic aorta by the method outlined above.

The examination of the distribution of the finer vessels within tissues or organs should preferably be undertaken after elucidation of the gross anatomy, thereby correlating the arterial supply and venous drainage. Some information may already have been obtained from specimens injected with Micropaque, but it is usually also necessary to utilize an injection medium which will flow easily through the capillary bed, such as india ink. The organ or tissue is then immediately fixed in formol saline, cleared and serial frozen sections, 50-100 μ thick, prepared. It may also be desirable to stain the sections lightly, in which case they must be made thinner (10-15 μ). If it is desired to radiograph the sections then a radiopaque medium which will easily fill the capillaries must be utilized: the most desirable substance from this point of view is Thorotrast (Testagar & Co., Inc.), a colloidal preparation of 24-26% thorium dioxide whose particles are of ultra-microscopic size. This medium has the additional advantages that it is non-toxic, freely miscible with blood

CHAPTER II

METHODS AND TECHNIQUE

THE injection of vessels supplying the adrenal gland is essential for a thorough investigation of their course and complexity, since the adrenal arteries are multiple and of fine calibre, and any attempt at their dissection without prior injection is both laborious and difficult. Of the various injection media utilized in this laboratory for observations on the gross anatomy of the circulatory system, the one found of greatest value is a stabilized micro-dispersion of barium sulphate (Micropaque; Damancy & Co. Ltd.). This has the advantage of penetrating into the finest branches of the arterial system and even into capillaries on occasion, but very rarely, if ever, through them into the venous system, when injected into a major artery; the particle size of Micropaque varies from 0.1-1 μ, yet the particles do not pass through capillaries whose diameter is approximately 10 μ, and this may be explained by the high viscosity of 50-100% Micropaque (Table I), which is much greater than that of blood. If, however, arteriovenous anastomoses, or vessels of larger than capillary size linking the arterial with the venous system (e.g. the

TABLE I

THE VISCOSITY OF MICROPAQUE SUSPENSION AT 20° C

Dilutions	*Dynamic viscosity in centipoises*	*Specific gravity*	*Kinematic viscosity in centistokes*
100% Micropaque	290	1 793	162
50% "	26	1 387	18 7
30% "	10	1 232	8 1
25% "	8	1 184	6 8
Pure water	1 005	0 998	1 007

Note 1 Viscosity will be lower still at body temperature (around 37° C)
2 The viscosity of blood is 3 5-5 4 times that of water.

juxta-medullary glomeruli of the kidney), are present and functioning, Micropaque may readily appear in the venous system draining an organ. Similarly, following retrograde injection into a large vein, this injection medium will penetrate (the absence of valves permitting) into the venous system and its ramifications only. By separate injections it is therefore possible to demonstrate only the arterial or venous supply of an organ to the exclusion of the other. Being radiopaque the vessels injected with Micropaque can also be demonstrated by radiography prior to dissection: when vessels ramify in different planes, this is often of great advantage. Finally, the vessels following injection

In the investigation of the adrenal circulation an additional requirement was necessary, for an injection medium which could be introduced into the general circulation, and whose course through the adrenal could be followed in the living animal in varying functional states over successive periods of time during the course of an experiment lasting up to 2 hours or more, and whose distribution within the vessels of the gland could be examined at the termination of the experiment. Thorotrast was found to be the most suitable medium, for the reasons mentioned above, since its course and ultimate distribution could be followed radiographically at periods up to 3 hours after injection. It was introduced through a cannula into a common carotid artery of the animals used, in quantities varying from 3-10 ml depending on the size of the animal. In larger animals (rabbit and monkey) the degree of filling of intraglandular vessels of the adrenal could be detected by the radiopacity of the gland on ordinary radiographic examination, since it was found (Harrison, 1957) that the opacity of the gland to X-rays in these animals increased with increasing filling of adrenal cortical capillaries. If intracortical vessels are not filled with radiopaque medium, the gland is not visible radiographically. In the rat, however, because of the smaller size of the gland, such methods were unsatisfactory. Nevertheless, in all animals, at any time during an experimental procedure, or at the termination of the experiment, it was possible to remove a gland from the anaesthetized animal within seconds and place it in 70-96% ethyl alcohol, which fixed the Thorotrast *in situ* exactly in the location it occupied at the moment of removal. By subsequent frozen sectioning of the gland, the resultant 100-250 μ sections (the thickness depending on the experimental animal) may be examined by radiographic methods

The radiographic examination of gross specimens (e.g. a whole rat, or an organ from it) is accomplished by routine methods. In this department a Watson portable Mobilix apparatus has been found to be quite satisfactory. For routine radiography, Ilfex (Ilford Ltd.) non-screen radiographic film, envelope packed, is used; an exposure of 0 2 second at 60-67 kV, 53-50 mA being adequate for a 300 g. rat. For a rabbit 0.3-0 4 second, and for a 3-6 kg. monkey 0 5 second, at the same kV and mA are necessary. Such radiographs are not suitable for enlargement: in fact, the maximum enlargement permitted by Ilfex film without the appearance of grain is 2-3 diameters. For the demonstration of finer detail in gross specimens, Kodaline film (Kodak Ltd., R O 5) is used. in these circumstances an exposure of 7-8 seconds at 60-67 kV, 53-50 mA is needed for a whole rat, and for its excised organs, such as the testis or spleen, 4 seconds at the same kV and mA The radiographs obtained on Kodaline film can be enlarged 6-8 diameters with excellent results. For the examination of smaller organs, such as sections of the adrenals of a rat, more specialized methods are necessary, since even after exposure on Kodaline

and other body fluids, and does not itself appreciably affect blood flow or pressure (the injection of 10 ml. Thorotrast intra-arterially in a mature rabbit produces a rise of blood pressure lasting only 20-30 seconds of the order of 10 mm. mercury), or the calibre of blood vessels. It is also markedly radio-paque. It can only be utilized in relatively acute experiments on animals, however, since it is taken up by the reticulo-endothelial system, particularly in the spleen, and may produce neoplastic growth there by means of its γ-radiation. Since Micropaque is a particulate dispersion it should also never be utilized for the injection of blood vessels in the living human, because of the danger of embolism.

When the gross vascular pattern of an animal or one of its organs is to be examined, it should preferably be injected immediately after the death of the animal or when the organ or tissue is removed immediately after death. In this laboratory the animal is killed by means of an overdose of chloroform or coal gas; in such a case no blood clotting sufficient to obstruct the filling of even finer blood vessels has been observed, but if it is impossible to inject the animal immediately after death, the ante-mortem injection of heparin (e.g. 1000 i.u. intraperitoneally in a 300 g. rat) will mitigate the post-mortem clotting and facilitate injection. The injection of an animal is made either into the descending thoracic aorta, the common carotid artery or the inferior vena cava after the insertion of a needle or polythene cannula, which is secured in place by a thread ligature around the vessel. For injection of the thoracic aorta the left side of the chest wall is first opened, and similarly the right side of the chest wall for the inferior vena cava. Injection can, of course, be made into any vessel, and either along the direction of blood flow or retrogradely. In the case of isolated organs the cannula is inserted directly into the vessel of choice. The largest bore needle or cannula which may be admitted by the vessel to be injected is advisable when using particulate media such as Micropaque, in order that it may not be obstructed. A syringe charged with injection medium may be utilized for the procedure, and injection carried out with a steady and even pressure. Such a pressure can also be achieved by an apparatus working on simple hydrostatic principles, or utilizing positive pressure from a pressure bulb or oxygen cylinder, and it is usually desirable in such cases to fit a manometric device so that injection can be made at known physiological pressures. With media of larger particulate size, a point of resistance is reached beyond which it is ill-advised to attempt further injection, otherwise vessels will burst, owing to the medium being held up at the capillary bed. It is often of great value to make an incision into the plantar aspect of the foot of a rat before injection: this prevents the attainment of high pressures in the vascular system and the occurrence of burst vessels. The vessel injected should be clamped and ligated following injection.

left adrenal was examined by radiography at intervals of 2 seconds to 5 minutes in the earlier experiments, but in later experiments this period was lengthened, often to as much as 30 minutes. When a radiograph was being taken, the spleen, stomach and intestines were removed from over the area of the left adrenal by means of retractors through an abdominal incision parallel to and about 1 in. below the left costal margin.

In three of the rabbit experiments, the control of the adrenal circulation was examined in decerebrate, hypophysectomized animals. This procedure was effected through a trephine hole in the calvarium after ligature of both carotid arteries high in the neck. All brain substance above the level of the pons was removed and the hypophysis cerebri quickly extracted by strong suction effected by a vacuum of 12.5 cm. mercury. Careful examination of the sella turcica after the conclusion of the experiments by means of a binocular stereoscopic microscope at a magnification of × 35 confirmed the effectiveness of this procedure. Such rabbits required artificial respiration through an intratracheal cannula by means of a respiratory pump during the remainder of the experiments.

In order to determine whether the findings in the preliminary experiments on the rabbit are also applicable to another mammal, further similar observations were carried out on five macaque monkeys, one male and four females, varying in weight from 3.5-6.22 kg. The details of the experimental procedure were exactly as for the rabbit.

It was soon realized, however, that more extensive examination of the adrenal circulation in large numbers of animals was necessary, in order that the time relationships and effect of functional variations on adrenal circulation could be examined more thoroughly. The rat was considered ideal since it is available in large numbers, but it has the disadvantage that its adrenals are too small to be examined by conventional radiographic methods during the course of an experiment. However, the adrenals could be removed at any time during the course of an experiment and examined by microradiographic methods by the technique outlined above, so providing an analysis of adrenal vascularization at varying intervals after the introduction of some functional alteration in the animal. Accordingly, in a further series of experiments, 175 male rats, varying in weight from 272-474 g. were used for observation of the effect of several physiological and pharmacological media and experimental procedures

The rats were anaesthetized with ether, injected intraperitoneally with 1000 i.u. heparin, a cannula inserted into the left common carotid artery and ligatured in position. 3-5 ml. Thorotrast were then injected slowly into the cannula. The experimental procedure (e.g. injection of adrenaline) was then undertaken, and the adrenals removed after a varying time period, fixed in

film subsequent enlargement reveals the grain of the film before details of intraglandular vessels are visible, an enlargement of more than 8 diameters being necessary. Therefore the technique of microradiography must be applied.

The first serious attempt at the visualization of fine vessels by radiographic methods in biological material following the injection of radiopaque media was made by Barclay (see Barclay, 1951). He realized that, in order to observe the details of such vessels, a film grain of sufficiently fine size is necessary in order that the radiograph obtained may be enlarged adequately, and a specialized type of X-ray apparatus is necessary in order to produce soft X-rays and expose the fine-grain emulsions for longer periods than are possible with the conventional type of radiographic machine. Such requirements are met by the various modifications of X-ray diffraction units now available, and the one employed in this laboratory is the Hilger microfocus X-ray unit incorporating the Ehrenberg and Spear tube. This unit has a tube which is continuously evacuated, and an anode which is cooled continuously by means of a very thin oil circulated by a pump. We use it with a Beryllium window in the tube, and Kodak Maximum Resolution plates, extremely fine emulsion plates which are capable of resolving at least 1000 lines per mm.

Since Barclay's pioneer investigations much subsequent research has been performed on the technique of microradiography (see *Kodak Bibliography on Microradiography and Soft-X-ray Radiography*, and in particular Bellman, 1953; Cosslett, Engström and Pattee, 1957). The classical method of micro-radiography (the 'contact method') consists in exposing the object (e.g. a section of adrenal), placed close to the fine-grain emulsion plate, to the X-rays from the tube which is at a distance, and subsequent enlargement of the plate ('secondary magnification'). This is the method we utilized, the tube filament-plate distance being 40 cm., and the exposure made at 40 kV, 300-400 mA for 10-15 minutes. It is also possible to utilize projection microradiography in which the object is placed close to the source of X-rays, using a fine focus tube, so obtaining primary enlargement ('primary magnification') of the object on the plate, but this method was not utilized in our experiments.

In the preliminary investigation of the functional control of adrenal vascularization (see Harrison, 1957) the rabbit was the animal of choice, since its adrenals are large enough to be visualized *in situ* by serial radiography on Ilfex film following the injection of 10 ml. Thorotrast into a common carotid artery, the axillary artery, axillary vein or a jugular vein — usually the first or the last. Eighteen rabbits of both sexes varying in weight from 1.8-3 7 kg. were used in the experiments and anaesthetized by intravenous nembutal and ether. Arterial blood pressure was measured by a mercury manometer attached to a cannula inserted into the other common carotid artery. The

CHAPTER III

THE ANATOMY OF ADRENAL VASCULARIZATION

The uniform feature regarding the vascularization of the adrenals of all mammals so far examined is the multiplicity of arteries supplying the gland, whereas there is only one, or at the most three, veins draining each adrenal. The right vein constantly drains into the inferior vena cava and the left into the left renal vein. In man the intraglandular part of the single adrenal vein has only longitudinal smooth muscle in its coat for the greater part of its length (Brunn, 1873; Ferguson, 1906; Bargmann, 1933; Velican, 1948; Heimvaara, 1954). Within the gland there is also a unique feature which has been found to occur in all mammalian adrenals investigated. This is the presence of an arterial supply to the medulla independent of the vascularization of the cortex, accomplished by means of the 'arteriae medullae' of Flint (1900) – medullary arteries which pass radially through the adrenal cortex to the medulla in centripetal fashion without giving off any branches to the cortex.

THE RAT

An arterial stem, taking origin from the ventral aspect of the aorta, is the main source of arterial supply of the left adrenal in the rat; it divides into two main arteries both of which give off branches to the cephalic aspect of the gland (Harrison, 1951b) The anterior of the two branches also supplies the inferior aspect of the diaphragm, and may therefore be considered as homologous with the human inferior phrenic artery. A second arterial stem arising from the ventral aspect of the aorta at a slightly more caudal level, also provides a branch to the medial aspect of the gland (Figs. 1 and 2). The left renal artery provides an inconstant adrenal artery to the inferior pole of the left gland. A similar arterial supply is provided to the right adrenal (Fig. 3), except that almost the whole vascularization is effected through the branches from the inferior phrenic artery One or two arteries may also be given off from the right renal artery to the caudal aspect of the gland. The venous drainage is by way of a single vein into the posterior vena cava on the right, and the renal vein on the left.

The intraglandular circulation in the rat consists of two types of vessels arising from a poorly defined capsular plexus (Gersh and Grollman, 1941):

96% alcohol, 250 μ frozen sections taken and subjected to microradiography. Five rats were hypophysectomized by means of the standard para-pharyngeal approach originally devised by Smith (1930) using an intratracheal cannula 2, 5 and 22 days before subjecting them to the investigation just outlined. As a safeguard against the occurrence of secondary infection, the hypophysectomized rats were injected intraperitoneally immediately post-operatively and again on the two succeeding days with 20 mg. Terramycin hydrochloride in 0.2 ml. pyrogen-free distilled water. The success of the operation was determined post mortem by removing the calvarium from the skull, lifting up the brain and exposing the pituitary, if present, using a binocular microscope, if necessary. In addition, the testes, adrenals and thyroids of such animals were weighed, examined histologically and compared with those of littermate rats. Unilateral (left) adrenalectomy was performed in some rats through a midline dorsal skin incision, and a muscle incision bisecting the left renal angle. In a further experimental series some rats were exposed to the stress of heat shock in a hot room, or to a low temperature in a refrigerator; details of these experiments will be given later.

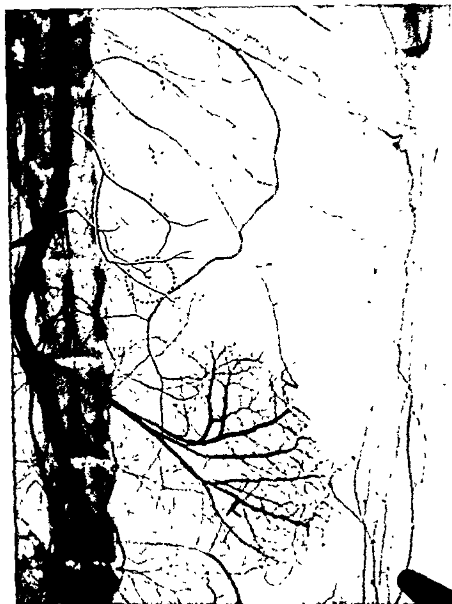


Fig 2 Arteriogram of the left adrenal and kidney of the rat The left adrenal has been outlined. The vessels supplying it have also been outlined to demonstrate them more clearly. The adrenal and kidney have been separated by pulling on adjoining tissues with forceps to make the detail obvious; hence the aorta appears kinked.

1. The arteriae medullae, which pass through the cortex without giving off any branches and empty into the medullary sinusoids.

2. The cortical circulation consisting of capillary sinusoids which first form an efficiently anastomotic network around the cells of the zona glomerulosa. From this network arise longitudinal capillary sinusoids which pass centripetally in between the columns of zona fasciculata cells and anastomose with each other very poorly; these then open into a plexus of sinusoids of larger bore and irregular outline in the zona reticularis, and these in turn empty into the medullary sinusoids which are drained by the central adrenal vein.

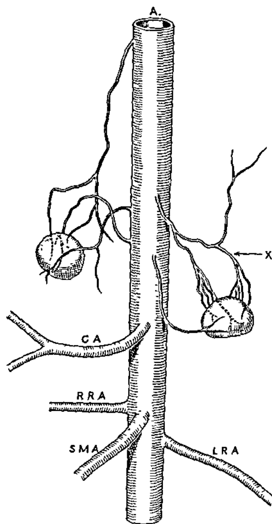


Fig. 1 Diagram of the arterial supply of the adrenals in the rat

A = Aorta, C A = Coeliac artery, L R A = Left renal artery, R R A = Right renal atery, S M A = Superior mesenteric artery

The arterial supply to the adrenal cortex is of great interest from the viewpoint of anastomotic efficiency. In a female rat weighing 190 g. one of the main arteries reaching the cephalic aspect of the gland was interrupted (at X in Fig. 1), and the animal killed 3 days later The resulting histological appearance of the gland demonstrates an area of focal necrosis involving approximately one-third of the zona fasciculata of the cortex (Fig. 4). Over the surface of this necrotic zone the capillaries of the glomerulosal network and a thin outer rim of zona fasciculata are distended with erythrocytes; the remainder of the cortex shows very pronounced hyperaemia and the cortical capillary sinusoids here are so distended with erythrocytes that localized cystic dilatations may be seen in the zona fasciculata (Harrison, 1951b). Individual adrenal arteries are therefore end-arteries to the zona fasciculata of the cortex. Since the medulla has a very efficient blood supply from two sources it is very unlikely that localized ischaemia of the gland would produce focal necrosis of the medulla; the efficient anastomotic

circulation in the zona glomerulosa and zona fasciculata also explains why these zones escape the focal necrosis.

Gersh and Grollman (1941) undertook an extensive investigation of the vascularization of the adrenal in the mouse and rat, and found that there are from one (rarely) to four arteriae medullae in each adrenal, arising most commonly in a part of the capsule far removed from the point where the central vein emerges. They found that the degree of injection of adrenal cortical



FIG 4 F' [illegible]
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capillaries is more complete in stimulated than in normal glands, and since there is no clear evidence of the existence of nerve terminations in cortical cells [illegible]

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in the rat adrenal cortex, and suggested that sudden alterations in cortical capillary flow are effected by nervous control of the calibre of the arteriae

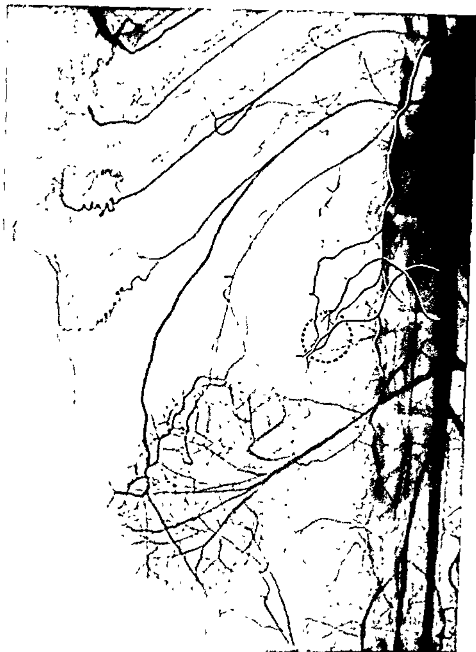


Fig 3 Arteriogram of the right adrenal and kidney of the rat prepared in the same way as Fig 2

circulation in the zona glomerulosa and zona fasciculata also explains why these zones escape the focal necrosis.

Gersh and Grollman (1941) undertook an extensive investigation of the vascularization of the adrenal in the mouse and rat, and found that there are from one (rarely) to four arteriae medullae in each adrenal, arising most commonly in a part of the capsule far removed from the point where the central vein emerges. They found that the degree of injection of adrenal cortical



FIG. 4. Photomicrograph of a histological section through the left adrenal of a rat following interruption of an artery supplying the gland (at × in Fig. 1). An area of focal necrosis in the adrenal cortex involving approximately ¼ of the zona fasciculata has been produced. The remainder of the cortex shows hyperaemia (× 18)

capillaries is more complete in stimulated than in normal glands, and since there is no clear evidence of the existence of nerve terminations in cortical cells, considered it profitable to regard the extreme plasticity of the capillary bed of the adrenal cortex as at least a partial mechanism for the control of its secretion.

Lever (1955) has recently investigated the cellular-vascular relationships in the rat adrenal cortex, and suggested that sudden alterations in cortical capillary flow are effected by nervous control of the calibre of the arteriae

corticis, *arteries passing to various depths in the cortex*, whereas a basic and slower control is due to the degree of capillary compression by cells of the zona intermedia (sudanophobe or transitional zone) and outer zona fasciculata *under the influence of the pituitary*.

Gersh and Grollman (1941) and Ezaki (1958) have investigated the blood supply of the mouse adrenal, and found close resemblances to that in the rat.

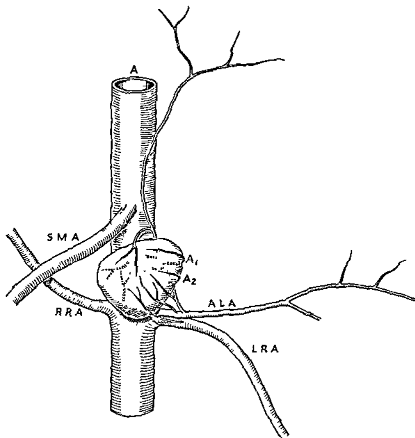


FIG 5 Diagram of the arterial supply of the left adrenal of a rabbit

A = Aorta, A_1, A_2 = Arteries curving around the left lateral border of the adrenal to supply it These arteries when interrupted produce an area of focal necrosis in the adrenal cortex A L.A = Adrenolumbar artery, L R A = Left renal artery, R R A = Right renal artery, S M A = Superior mesenteric artery

THE RABBIT

Since observations on the functional control of cortical vascularization in this animal were confined to the left gland, the anatomy of the blood supply on this side only was investigated (Harrison, 1951b). The left adrenal lies in the angle formed by the aorta and left renal artery and is provided with arteries mainly derived from the adrenolumbar artery, which arises from the aorta just above the left renal artery, or from the renal artery itself (Fig. 5). A

branch of the adrenolumbar artery passes cephalically, deep to the gland, and provides five to nine branches to the left half of the adrenal. One or two adrenal arteries arise from the left renal artery or adrenolumbar artery before giving off the adrenal branch which, because it ends by supplying the diaphragm may be considered as an inferior phrenic artery. Two or three arteries arising directly from the aorta supply the right half of the gland from its deep surface or medial border. There is only one adrenal vein, as in the rat.

The finer branches of the adrenal arteries ramify in the superficial part of the capsule of the gland, anastomosing very poorly with one another. These vessels then form a subcapsular arteriolar plexus from which both arteriae medullae and cortical capillaries arise. The latter form a glomerulosal network which gives origin to zona fasciculata capillaries; the latter anastomose poorly by sparse cross-communicating capillaries. In the zona reticularis the capillaries again form a plexus, and eventually open into the medullary sinusoids.

Occasionally direct anastomoses of branches of arteries on the anterior surface of the gland with small venous radicles draining tissues around the gland may be observed. Bennett and Kilham (1940) noted four or five such anastomoses in the connective tissue around a single adrenal in the cat.

Individual adrenal arteries in the rabbit are end-arteries to the zona fasciculata of the cortex (Harrison, 1951b). Interruption of a single artery arising from the adrenal branch of the adrenolumbar artery produces an area of focal necrosis which may be detected as early as 3 days after operation. Four days after operation (Fig. 6) the lesion is very noticeable. Degeneration in the area of focal necrosis is pronounced 2 and 3 weeks after operation (Fig. 7), while after 4 or 5 weeks the area of focal necrosis is visible as a triangular lesion depressed from the surface of the cortex involving only the zona fasciculata, the zona glomerulosa and zona reticularis in relation to the lesion are relatively unaffected.

THE CAT AND DOG

The arterial supply of the cat adrenal is richer than in the rat and rabbit since twenty or more arteries enter the gland around its periphery mainly from the renal artery, but also from the adrenolumbar, coeliac, phrenic and, occasionally, the superior mesenteric artery, as well as the aorta (Bennett and Kilham, 1940; Harrison, 1951b). Also, in contrast to the rat and rabbit, these arteries form a rich anastomotic plexus in the capsule. Nevertheless, individual adrenal arteries are end-arteries to the cortex.

The vascularization of the dog adrenal was described in the classic paper of

Flint (1900). The adrenal is supplied by eleven to twenty-one arteries from the phrenic artery, aorta, renal artery, adrenolumbar artery and, inconstantly, from the coeliac and superior mesenteric arteries. The capsular plexus of arteries is *not definite and well marked; from it arise the vessels which break* up into capillaries and ramify in between the cortical cells. The anastomoses between zona fasciculata capillaries are frequent. Flint estimated that there



FIG. 6. Photomicrograph of the area of focal necrosis in the adrenal cortex of a rabbit four days after division of an artery supplying the left adrenal. The capsule is to the left of the figure and the focal necrosis can be seen to involve only the z. fasciculata (× 95).

are some 580 arteries in the capsular plexus of the adrenal of an average-sized dog; of these about fifty turn abruptly to pass through the cortex as arteriae medullae. There are two to four veins draining the medulla to empty into the adrenolumbar vein. There is also a venous plexus in the capsule passing into venae comites of the adrenal arteries, branches of the renal, phrenic and adrenolumbar veins. More recently Brondi and Castorina (1953) have

confirmed this blood supply, except that they claim the anastomoses between capillaries in the zona fasciculata to be poorly defined.

THE MACAQUE MONKEY

Both adrenals of the macaque monkey (*Macaca mulatta*) receive many small arteries forming a rich network around the periphery of the glands (Harrison and Asling, 1955). The left adrenal is supplied by arteries from three sources. A trunk arises from the coeliac artery to supply two or three arteries to the



Fig 7 Photomicrograph of the area of focal necrosis in a rabbit adrenal cortex two weeks after division of an adrenal artery The capsule is at the top of the figure The focal necrosis can again be seen to involve only the z fasciculata (× 95)

supero-medial aspect of the gland, and then divides into an inferior phrenic and an adrenolumbar artery (Fig. 8). The adrenolumbar artery supplies four vessels to the superficial and deep surfaces of the gland, one of them continuing as a renal capsular artery The aorta furnishes two arteries to the medial aspect of the adrenal, and four arteries arise from the left renal artery to supply the infero-medial aspect of the gland.

The right adrenal has superficial and deep sources of glandular arteries. The superficial supply consists of two arteries. One arises from the aorta at the level of the right renal artery, passes posterior to the inferior vena cava, anterior to the right renal vessels and the two right adrenal veins to furnish

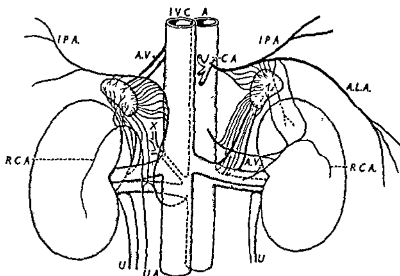


FIG 8 A diagram showing the arterial supply of the adrenals of a macaque monkey. The whole of the arterial supply to the left adrenal is shown, but only the superficial arterial supply to the right adrenal is depicted The deep arterial supply to the right adrenal arises from stem X shown in the figure

A = Aorta, A L A = Adrenolumbar artery, A V = Adrenal veins, C A = Coeliac artery, I P A = Inferior phrenic artery, I V C = Inferior vena cava, R C A = Renal capsular artery, U = Ureter, U A = Ureteric artery

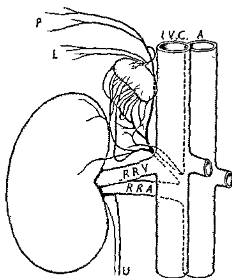


FIG 9 The deep arterial supply to the right adrenal arising from stem X shown in Fig 8

A = Aorta, I V.C = Inferior Vena Cava, L = Lumbar arteries, P = Phrenic arteries, R R A = Right renal artery, R R V = Right renal vein, U = Ureter

fifteen arteries to the medial and superior aspects of the gland. The second artery arises from the renal artery near the hilus of the right kidney and supplies four to five arteries to the adrenal. The deep arterial supply is provided by an arterial stem (X in Fig. 8) arising in the angle between the aorta and right renal artery; this breaks up into three main vessels which then supply thirteen to fifteen vessels to the gland (Fig. 9).

The adrenal arteries form a subcapsular network from which capillaries are given off to form a rich glomerulosal network, which then gives off capillaries in centripetal fashion towards the medulla (Fig. 10). Arteriae corticis (Fig. 11) and arteriae medullae (Fig. 12) may also be observed.

Arteries to the adrenal are end-arteries to the cortex. Five, 7 and 21 days after



[illegible] of the adrenal cortex of a macaque monkey [illegible]



Fig 11 Photomicrograph of the system of vessels in the adrenal cortex of a macaque monkey as seen following the injection of india ink An arteria corticis is seen dipping down into the cortex below the capsule, on the left of the figure (× 50)

interrupting adrenal arteries there is an area of focal necrosis invariably involving the zona fasciculata and perhaps also the zona glomerulosa and zona reticularis (Fig. 13).

MAN

Gérard (1913) showed that the human adrenal receives a number of arteries around its periphery from the classic three sources — the inferior



FIG 12 An arteria medullae in the adrenal cortex of a macaque monkey displayed by photomicrography in an adrenal whose arterial circulation has been injected with india ink (× 50)



Fig 13 An area of focal necrosis, involving only the z fasciculata, produced in the adrenal cortex of a macaque monkey by the interruption of a single adrenal artery The area of focal necrosis clearly involves only the z fasciculata, the z glomerulosa and z reticularis being unaffected (× 39)

phrenic artery, aorta and renal artery Pick and Anson (1940) studied the inferior phrenic artery particularly, and found that it arises most commonly from the coeliac artery; a direct aortic origin is less frequent They found that a number of superior adrenal arteries arise from this artery (Fig. 14). Anson, Cauldwell, Pick and Beaton (1947) later showed that a total of fifty or more arteries enter the periphery of the gland; in some cases their origin is chiefly from the inferior phrenic artery, while in others it is mainly from the renal

artery. Gagnon (1957) has made a detailed study of the origin, diameter and number of adrenal arteries. There is only one main adrenal vein on each side, however, draining into the inferior vena cava on the right, and the renal vein

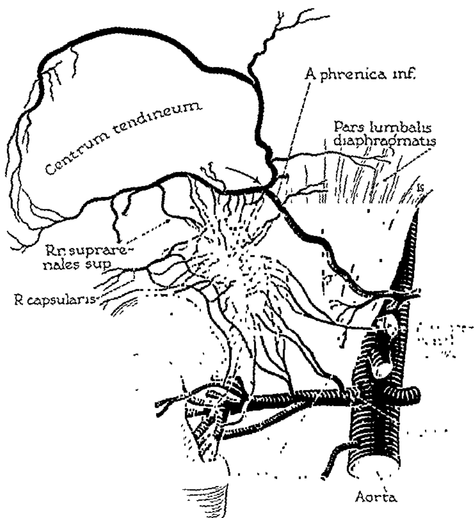


Fig. 14 The arterial supply of the human right adrenal gland. A large number of arteries reach the periphery of the gland from three sources — the inferior phrenic artery (Rr. suprarenales sup.), the aorta (A. suprarenalis med.) and renal artery (Aa. suprarenales inf.). (From Pick and Anson, 1940.)

on the left. Busch (1954) has also studied the distribution of arteries to the gland with particular reference to their origin and the anomalies to be encountered.

Gagnon (1956) in an exhaustive study of the venous drainage of the human

interrupting adrenal arteries there is an area of focal necrosis invariably involving the zona fasciculata and perhaps also the zona glomerulosa and zona reticularis (Fig. 13).

MAN

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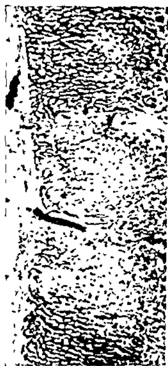


Fig 12 An arteria medullae in the adrenal cortex of a macaque monkey displayed by photomicrography in an adrenal whose arterial circulation has been injected with india ink (× 50)



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adrenal gland showed that the central vein is a very constant structure, although rarely being double, but stressed the importance of the numerous venae comites of the adrenal arteries, which may be utilized as collateral pathways following ligation of the central vein. Weisz and Torchiana (1956) also stress the variable course and surgical importance of these periadrenal veins. Johnstone (1957) claims that the right adrenal vein may drain into a

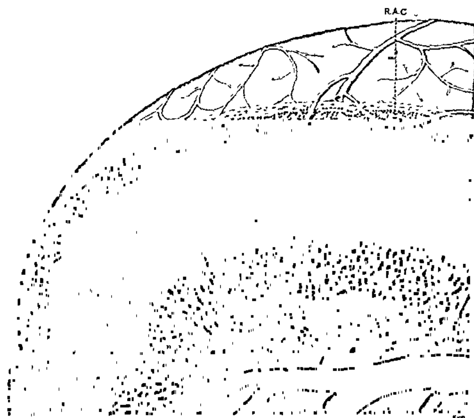


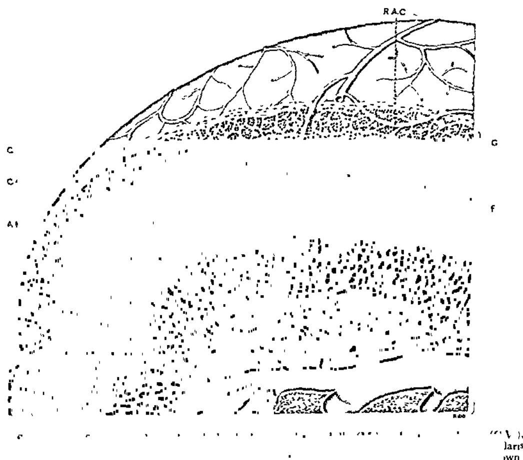
FIG 15 Stereogram of a mammalian adrenal gland, showing the medulla (M) with its central vein (C V), and the cortex with its three zones, the zona glomerulosa (Z G.), zona fasciculata (Z F) and zona reticularis (Z R), enclosed by the capsule (C) Two arteriae medullae (A M) and an arteria corticis (R A C) are shown The columns of cortical cells are mostly separated by capillary sinusoids (C C) (From Harrison, 1959)

right hepatic vein and may be double or triple, whereas the left adrenal vein invariably joins the left renal vein

The structure and intraglandular vascularization of the mammalian adrenal is depicted diagrammatically in Fig 15 It may be noted from this figure that blood reaching the periphery of the gland has two possible routes towards the medulla in centripetal fashion — the arteriae medullae and the multitude of

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Th ... ation of the mammalian adrenal ... ay be noted from this figure that ... two possible routes towards the ... medullae and the multitude of

In five other rabbits, however, rapid intramuscular injection of 1 ml. 1/1000 adrenaline tartrate solution (270-556 μg./kg.) resulted in both the adrenal and its vessels becoming very conspicuous radiographically after 1½ hours. Microradiography of a section of such an adrenal showed pronounced and complete filling of all intracortical vessels. An additional intramuscular injection of adrenaline tartrate appeared to hasten the onset of the filling of these vessels; thus when 0.45 ml. 1/1000 solution (214 μg./kg.) was injected into a rabbit weighing 2.1 kg, and followed in 28 minutes by a further 1.3 ml. (619 μg./kg.) filling of intracortical capillary sinusoids occurred within 5 minutes, i.e. 33 minutes after the first injection (Figs 17 and 18).

Adrenaline hydrochloride is not as effective in this mechanism; thus 1 hour 56 minutes after the intramuscular injection of 1 ml. adrenaline hydrochloride (455 μg./kg.) into a 2.2 kg rabbit, there was only slight filling of vessels in the outer one-third of the adrenal cortex (Fig. 18), an appearance which does not differ significantly from that in the control animal. A second injection, however, facilitated the filling of cortical vessels; thus, an intramuscular injection of 1 ml. adrenaline hydrochloride (270 μg./kg.) into a 3.7 kg rabbit followed 1 hour 47 minutes later by a further 1 ml. injected intravenously over a period of 12 minutes, produced marked filling of cortical vessels 9 minutes after the onset of the intravenous injection.



FIG 17 Radiograph of the area of the left adrenal of the same rabbit as in Fig 16, 33 min after the intramuscular injection of 0 45 ml 1/1000 adrenaline tartrate and 5 min after injection of 1 3 ml 1/1000 adrenaline tartrate The radiograph was taken 1 hr 10 min after that shown in Fig 16 without any further injection of Thorotrast Note the calibre of the posterior vena cava, aorta and superior mesenteric artery as compared with the same vessels in Fig 16

l a = Left adrenal made visible by the filling of its vessels with thorotrast, l al v = Left adrenolumbar vein, l r v = Left renal vein, s = Spleen, s m a = Superior mesenteric artery

The intramuscular injection of 1 ml adrenaline tartrate or hydrochloride causes a rise of blood pressure in about 2 minutes, of the order of 20 mm. Hg., and maintained for 15-20 minutes (Fig. 19). The injection of 1 ml. adrenaline hydrochloride (370 μg /kg.) intravenously in a 2.7 kg. rabbit over a period of 15 minutes, and after 2½ hours a further injection of 1 ml. intravenously over a period of 30 minutes, did not cause any filling of cortical vessels at any time up to 1 hour after the beginning of the last injection, i.e. 3½ hours after the

THE EFFECT OF ADRENALINE ON ADRENAL CORTICAL VASCULARIZATION

IN the earlier experiments on the rabbit (Harrison, 1957) adrenaline seemed to be the agent of choice for constricting the arteriae medullae and thereby diverting blood flowing through the gland into the cortical capillary sinusoids. The injection of 10 ml. Thorotrast into the vascular system of five anaesthetized, but otherwise normal, control rabbits displayed the blood vessels in the area of the left adrenal by routine methods of radiography, but serial radiography at intervals up to 3½ hours after the intravascular introduction of Thorotrast *failed to display periglandular or intraglandular adrenal vessels.* Rapid removal of the gland at intervals during the 3½ hours, and microradiography of frozen sections of the gland, revealed only a few intracortical vessels (Fig. 16).

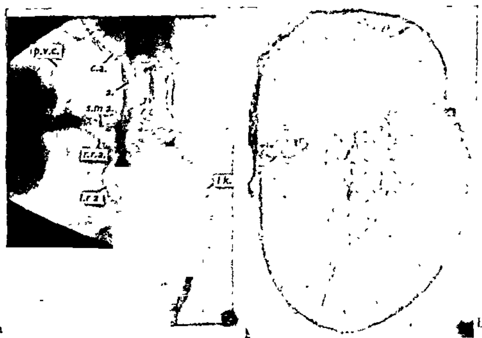


FIG. 16 (a) *Blood vessels in the region of the left adrenal* of a 2 1 kg male rabbit as shown by radiographs immediately after the injection of 10 ml. thorotrast into the right axillary vein The adrenal is not visible on the radiograph
a = ... l k = Left kidney l r a = Left renal artery, p v c = Posterior vena c ...
(b) ... min after injection of 10 ml ... ls are injected (× 10)

since the tartrate has a higher molecular weight than adrenaline hydrochloride, the amount of free adrenaline released from the tartrate would be smaller and the blood level of free adrenaline lower, than that from an equal quantity of hydrochloride, particularly after intramuscular injection. Again, because of the greater stability of the tartrate *in vitro*, it may be assumed that its duration of effect would be greater than that of the hydrochloride. Since intramuscular injection of the tartrate has the most marked effect in increasing the filling of intracortical capillary sinusoids, it therefore follows that lower levels of free adrenaline in the blood are more effective than higher concentrations in constricting the arteriae medullae. This may be explained by

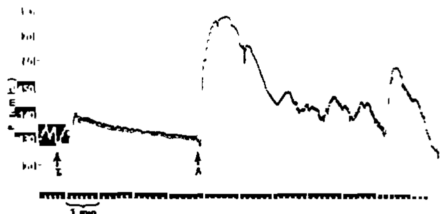


FIG. 20 Arterial blood pressure of a rabbit during injection of 10 ml. Thorotrast (at T) into the right internal jugular vein, and the first half of the record during the intravenous injection over a period of 15 min. of 1 ml. 1/1000 adrenaline hydrochloride (commencement of injection shown at A) into the right internal jugular vein.

several factors. Thus Bülbring and Burn (1942) have shown that, whereas small amounts of adrenaline augment the transmission of impulses in sympathetic ganglia, large amounts depress it; it is therefore possible that, apart from its direct vasoconstrictor effect on arteriae medullae, adrenaline may also facilitate the vasoconstrictor effect of impulses in the splanchnic nerves on these arteries. Such nerves supplying the arteriae medullae would be postganglionic, in contrast to those passing on to medullary cells, which are preganglionic. Nerve fibres have, in fact, been observed ending in relation to the arteriae medullae (Sarter, 1954). Secondly, adrenaline introduced directly into the circulation, or in higher doses by whatever route, may have a vaso-

Fig 18 (a) Microradiograph of a frozen section of an adrenal removed from a 2 2 kg male rabbit 1 hr 56 min after intramuscular injection of 1 ml 1/1000 adrenaline hydrochloride Only vessels in the outer one-third of the cortex are filled The adrenal vein (a v) is a prominent feature The periadrenal fat, seen in the lower left-hand corner of the figure, is well filled with Thorotrast (× 10)

(b) Microradiograph of a frozen section of the adrenal shown in Fig 17 Note the pronounced filling of cortical capillaries (× 10)

onset of the first injection Such an intravenous injection causes an immediate rise in blood pressure of almost 50 mm. Hg., and it is difficult with a protracted intravenous injection to maintain the pressure at a steady level (Fig 20). This difference in level of blood pressure is taken as reflecting differences in blood concentration of the hormone, intramuscular injection producing a lower level than intravenous infusion. This is probably caused in large part by the local vasoconstrictor effect of adrenaline at the site of intramuscular injection, thus delaying its entry into the general circulation. Also,



Fig 19 The effect on the arterial blood pressure of the rabbit of intramuscular injection of 1 ml 1/1000 adrenaline tartrate (at A in the figure)

microradiography of sections of the adrenals (Fig. 22). In another 6.22 kg. female macaque monkey 3 ml. 1/1000 adrenaline tartrate injected intramuscularly (482 µg./kg.) did not make the intracortical blood supply evident on radiography at any time up to 32 minutes after injection, but 6 minutes after intramuscular injection of a further 3 ml. adrenaline they were clearly visible (Fig. 23); microradiography of sections of the adrenals from this



FIG. 22. Microradiograph of a frozen section through the left adrenal of the monkey, whose radiograph is shown in Fig. 21 34 min. following intravenous injection of adrenaline tartrate. The intracortical vessels are not filled, although a few medullary veins are visible (in the lower left quadrant of the section) (× 10)

monkey revealed the presence of intracortical haemorrhages in approximately 1/3 of the circumference of the cortex (Fig. 24), a phenomenon which will be referred to later (p. 67).

The fact that intravenously administered adrenaline in small doses is without significant effect on adrenal ascorbic acid concentration in rats, whereas it has repeatedly been demonstrated that it is effective when administered by other routes, e.g. intramuscular and intraperitoneal, has been noted by

on the aorta and posterior vena cava with doses of the order of 1 ml. 1/1000 adrenaline tartrate injected intramuscularly. Only when the arteries supplying the adrenal dilate again and allow adrenaline to enter the gland would it be possible for it to exert an effect on the arteriae medullae.

The fact that intravenous injection of adrenaline has no effect on the blood



[illegible] pole of the left kidney in a macaque monkey

supply through the adrenal cortex was confirmed by an experiment on a 3.48 kg. female macaque monkey One ml. 1/1000 adrenaline tartrate solution (287 μg./kg.) had no effect in filling adrenal cortical capillary sinusoids as seen by conventional radiographic methods within 34 minutes (Fig. 21) and at the end of this time no radiopaque medium was visible in them by

intravascular injection of Thorotrast is valueless in the rat, since the arteries supplying the rat adrenals are larger in relation to its size, thereby masking the intrinsic blood supply of the cortex, microradiography of sections of the adrenals provides information of great value following experiments on this animal.

The standard experiment in the rat involved intramuscular injection of

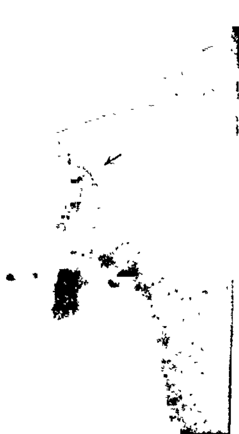


FIG. 24. Microradiograph of the left adrenal shown in Fig. 23. Intracortical haemorrhages have occurred in approximately one-third of the cortex (in the upper part of the figure) (× 11)

adrenaline tartrate 1/1000 solution after the injection of Thorotrast into a common carotid artery. In nine experiments on rats varying in weight from 275-474 g., a standard amount (0.2 ml.) of adrenaline was injected (422-727 μg./kg.), and the animals maintained under ether anaesthesia for 8, 10, 15, 30, 45, 50 and 60 minutes when the adrenals were removed. The results of these experiments demonstrate that there is no filling of cortical capillaries until

Munson and Briggs (1955). These workers, as others, interpret alterations in adrenal ascorbic acid concentration in the normal (non-hypophysectomized) rat as reflecting variations in ACTH secretion.

These two experiments on macaque monkeys confirmed that some modification of adrenal cortical blood supply could be achieved following intramuscular



[illegible]

injection of adrenaline, but further demonstrated that in order to observe the functional control of cortical vascularization more adequately, larger numbers of observations would be necessary Such observations would be impractical for financial reasons in the monkey, and therefore the use of some other smaller, and relatively less expensive, laboratory animal had to be contemplated. Although serial gross radiography of intracortical blood supply following

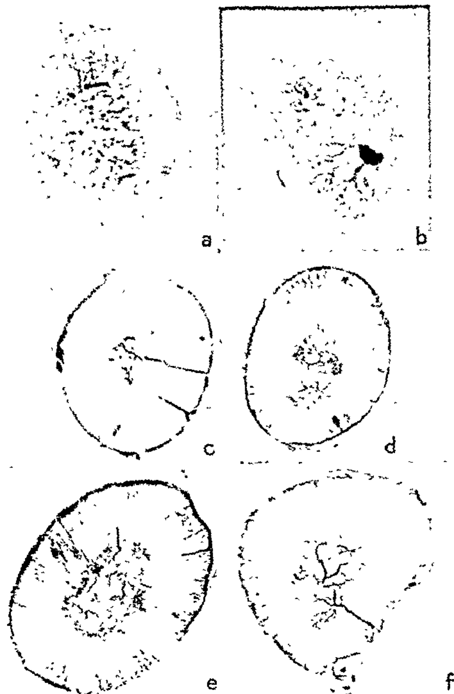


Fig. 27 Microradiograph of a section from an adrenal of a rat 4 min after the intra-arterial injection of 0·2 ml adrenaline tartrate 1/3000 solution (a) compared with others taken from control rats (b-f). The microradiograph in (b) was taken from the adrenal of a rat 30 min after the injection of 3 ml Thorotrast into a carotid artery. In both (a) and (b) the Thorotrast is visible in the medullary sinusoids and also fills the capillaries of the z. reticularis, although no radiopacity may be seen in either z. glomerulosa or z. fasciculata. It can only be presumed that the medullary sinusoids have been filled by arteriae medullae, and the z. reticularis by reflux from the medulla. The appearance in (c) was obtained by the injection of Micropaque into a normal control animal and shows the arteriae medullae very clearly. The control adrenals shown in (d) and (e) were examined 40 and 60 min respectively after the injection of 4 ml Thorotrast into a carotid artery. Arteriae medullae are not visible in (d), there being only a patchy filling of the outer z. fasciculata, but they are clearly visible in (e) — at approximately 2, 10 and 11 o'clock in the figure. The adrenal shown microradiographically in (f) was obtained immediately after the [illegible]al injection of Micropaque. One arteria medullae is clearly shown (at approximately 4 o'clock in the [illegible] outer z. fasciculata capillaries have been injected (a-d × 12, e and f × 14).

some 45 minutes after the injection. At this time, and at intervals up to 1 hour after the injection, there is pronounced filling of the cortical vessels (Figs. 25 and 26). *In two rats, 0.2 ml. adrenaline tartrate 1/3000 solution* (275-305 µg./kg.) was injected into the common carotid artery, and the adrenals removed 3 and 4 minutes later. No filling of adrenal cortical vessels significantly different from the normal control (see p. 53) could be observed, only the vessels in the inner zona fasciculata being filled (Fig. 27). A rat injected intra-



Fig 25 Microradiograph of a frozen section of a rat adrenal cortex 45 min after the intramuscular injection of 0·2 ml 1/1000 adrenaline tartrate solution All cortical capillaries are well injected particularly those in the z. glomerulosa The medullary sinusoids are empty (× 16)

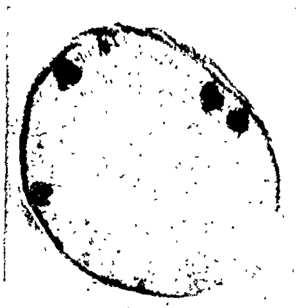


Fig 26 Microradiograph of a frozen section of an adrenal from another rat 45 min after the intramuscular injection of 0·2 ml 1/1000 adrenaline tartrate Haemorrhages have occurred into the outer z fasciculata in four places *The medullary sinusoids have not been filled* (× 20).

arterially with 0.1 ml. 1/1000 adrenaline tartrate solution (400 µg./kg.), however, showed filling of adrenal cortical vessels after 35 minutes.

The effect of *intraperitoneal* injection of adrenaline on cortical vascularization was investigated by injecting 0.2 ml. 1/1000 adrenaline tartrate solution (526-656 µg./kg.) in four rats varying in weight from 305-380 g After 37, 45 and 50 minutes the adrenal cortical capillaries were clearly obvious microradiographically, except that those in the outer zona fasciculata were not as *completely filled as those in the* zona glomerulosa and inner zona fasciculata.

It was considered that these experiments demonstrate clearly that adrenaline

THE EFFECT OF OTHER AGENTS WHICH ALTER CALIBRE OF BLOOD VESSELS ON CORTICAL VASCULARIZATION

IN the rabbit experiments (Harrison, 1957) histamine was found to be effective *in producing filling of adrenal cortical vessels*; *1 hour* after intravenous injection of 2 mg. histamine acid phosphate (B.D.H.) into a 2.4 kg. female rabbit the adrenal vessels were evident radiographically (*Fig. 28*) as *well* as

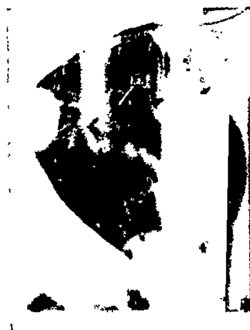


FIG 28 Radiograph of the left adrenal (l a) of a 2 4 kg female rabbit 1 hr after the intravenous injection of 2 mg histamine and acid phosphate intravenously



FIG 29 Microradiograph of a section of the adrenal shown in Fig 28, removed 3 hr after the injection of histamine (× 10)

microradiographically (Fig 29). In an experiment on a 4 3 kg. male macaque monkey 1 ml. 1/1000 adrenaline tartrate solution (234 μg./kg.) injected intramuscularly followed by a further 1 ml. in 10 minutes failed to produce radiographic evidence of adrenal cortical filling during the *succeeding 80 minutes*; then a further 1 ml adrenaline tartrate injected intramuscularly failed to

injected intramuscularly, intraperitoneally or intra-arterially, has a profound effect on adrenal vascularization in the rat causing filling of cortical vessels 35 minutes or more after injection. It was decided, therefore, that the effect of other substances altering the calibre of blood vessels, both vasoconstrictors and vasodilators, should be determined, since it is presumed that the influence of adrenaline is exerted by way of its vasoconstrictor effect on the arteriae medullae, and that it would be important to determine whether this effect is specific for adrenaline.

supply achieved by histamine may therefore be secondary to cellular changes in the cortex. On the other hand, the rapidity with which the increased vascularization of the adrenal cortex was effected in the monkey experiment, as compared with that in the rabbit, may suggest that a prior conditioning of the adrenal cortical blood supply by adrenaline may facilitate the action of histamine.

Munson and Briggs (1955) have already noted that histamine acts more rapidly than adrenaline, and conclude that the influence of histamine on adrenal ascorbic acid concentration cannot be mediated by adrenaline.

Since adrenaline is presumed to exert its effect by virtue of a vasoconstrictor action on the arteriae medullae, it was considered desirable that the action of other vasoconstrictor substances on the adrenal cortical blood supply

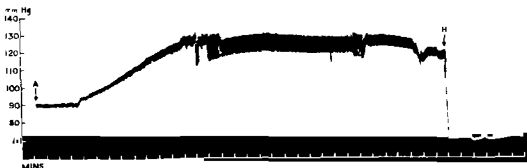


FIG 31 Arterial blood pressure of a 4 3 kg male macaque monkey following a third injection of 1 ml 1/1000 adrenaline tartrate solution (at A in the figure), showing the profound fall in pressure occasioned by intravenous injection of 1 mg histamine acid phosphate (at H in figure).

of the rat should be determined. Noradrenaline was clearly the first choice, since it is a natural product of the adrenal medulla, and also acts as a vasoconstrictor (von Euler, 1956). The noradrenaline utilized was Levophed (Bayer); 2 mg. in 2 ml. of this solution was diluted with normal saline to a volume of 200 ml.; 0 2 ml. (=2 μg noradrenaline) of this solution was injected intra-arterially into a common carotid artery in two rats, weighing 355 and 380 g. respectively (5 6 and 5 3 μg /kg. respectively), and the animals killed after 30 and 60 minutes Two other rats, weighing 320 and 415 g. respectively, were given intramuscular injections of 0 2 ml. of the diluted solution (6.3 μg. and 4.8 μg /kg of noradrenaline respectively) and killed after 30 and 60 minutes. After intra-arterial injection of noradrenaline filling of the cortical vessels occurred after both 30 and 60 minutes, whereas following intramuscular injection filling was observed after 60 minutes but not after 30 minutes (Fig. 32).

provide radiographic evidence of filling of intracortical vessels at intervals during the next 32 minutes. At this time 1 mg. histamine acid phosphate injected intravenously produced radiographic and microradiographic (Fig. 30) appearances suggesting adrenal cortical filling in 16 minutes. This experiment further suggests that the dose of adrenaline tartrate necessary to occasion adrenal cortical vascular response, even when repeated, is rather critical. The



FIG. 30. Microradiograph of a section of an adrenal removed from a macaque monkey 27 min. after intravenous injection of 1 mg. histamine acid phosphate preceded by intramuscular injections of 1/1000 adrenaline tartrate. Vessels of the z. fasciculata throughout the cortex have been injected. During the 11 min. prior to removal of the gland gross radiographic evidence of filling of the intraglandular vessels was clearly present (× 10).

histamine, as might be expected, occasioned a pronounced fall of the high blood pressure (Fig. 31), previously produced by the adrenaline. This effect of histamine is probably exerted directly on the adrenal, since it causes cortical secretion under the highly artificial conditions (see Harrison and Asling, 1955) of perfusion of the isolated gland (see Bibliography) and may act directly upon the adrenal cortical cell, by altering capillary permeability or causing capillary dilatation within the adrenal cortex. The alteration in intracortical blood

Adrenochrome, the yellow pigment produced in the adrenal gland, also exerts a vasoconstrictor effect. Lecomte and Fischer (1951) showed that it sensitizes the nictitating membrane of the cat to the action of adrenaline and in order to determine whether or not the adrenal gland is sensitized to the action of adrenaline in the same way, or whether adrenochrome has a direct effect on adrenal cortical blood supply, two series of experiments were carried out:

(i) Four rats varying in weight from 280-385 g. were each given an intramuscular injection of a solution of adrenochrome monosemicarbazone dihydrate (Adrenoxyl: Horlicks Ltd.) containing 2.5 mg. in 100 ml. distilled water. Three of these received 50 µg. adrenochrome (130-161 µg./kg.) in 0.2 ml. distilled water, and were killed after 30 and 60 minutes; the fourth, weighing 280 g., was injected with 0.15 ml. (37.5 µg.) of solution (134 µg./kg.) and killed after 60 minutes.

(ii) Intraperitoneal injections of 0.2 ml. of the same solution were given to four rats of weights varying from 287 to 460 g. (109-174 µg./kg.). The experiments were terminated after 20, 32, 45 and 60 minutes.

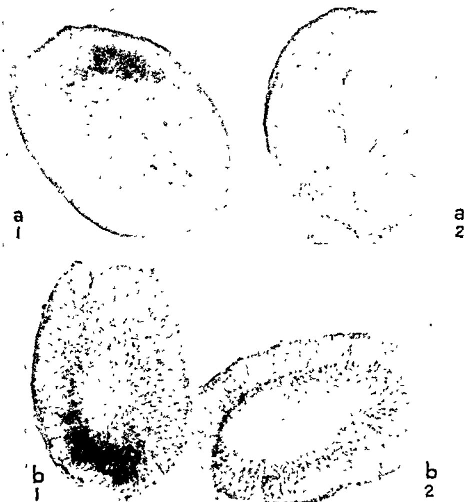


FIG. 33. The filling of adrenal cortical vessels shown by microradiography 60 min. after the intramuscular injection of 50 µg. serotonin, in 0.2 ml. solution into a rat. Vessels of the z. fasciculata are filled, and those of the z. reticularis are particularly noticeable. The medullary sinusoids are empty (× 12)

In all the above experiments, filling of adrenal cortical capillaries was noted after 30 minutes or more following injection, but not after 20 minutes.

Kallidin is a vasodilating agent released on dilution of serum or plasma (Schachter, 1956) and when serum comes into contact with glass (Armstrong *et al.*, 1953). Since histamine, another vasodilator, alters adrenal cortical vascularization, it was decided to examine the effect of ox kallidin on adrenal cortical blood supply. Four experiments were performed on rats varying in weight from 330-400 g. Each rat was injected intramuscularly with 250 µg. ox kallidin dissolved in 0.2 ml. distilled water (or, in one experiment 0.05 ml. water). The rats therefore received 625-689 µg./kg. ox kallidin and were killed after 10, 17, 40 and 60 minutes. Only in the 60-minute experiment was there slight filling of the intracortical vessels, and no filling at all in the other experiments.

Serotonin (5-hydroxytryptamine) has a well-marked vasoconstrictor effect on pulmonary and renal vessels (Page, 1958) when large doses are given quickly. Three experiments were therefore performed on rats weighing 355, 365 and 400 g. Each was injected intramuscularly with 50 µg. serotonin in



0.2 ml. of distilled water (141, 137, and 125 µg./kg. respectively), and the rats killed after 20, 40 and 60 minutes when the adrenals were removed. Filling of adrenal cortical vessels was only observed after 60 minutes (Fig. 33) and not after 20 or 40 minutes

Adrenochrome, the yellow pigment produced in the adrenal gland, also exerts a vasoconstrictor effect. Lecomte and Fischer (1951) showed that it sensitizes the nictitating membrane of the cat to the action of adrenaline and in order to determine whether or not the adrenal gland is sensitized to the action of adrenaline in the same way, or whether adrenochrome has a direct effect on adrenal cortical blood supply, two series of experiments were carried out:

(i) Four rats varying in weight from 280-385 g. were each given an intramuscular injection of a solution of adrenochrome monosemicarbazone dihydrate (Adrenoxyl: Horlicks Ltd) containing 2 5 mg. in 100 ml. distilled water. Three of these received 50 µg. adrenochrome (130-161 µg./kg.) in 0.2 ml. distilled water, and were killed after 30 and 60 minutes; the fourth, weighing 280 g., was injected with 0.15 ml (37.5 µg.) of solution (134 µg./kg.) and killed after 60 minutes.

(ii) Intraperitoneal injections of 0.2 ml. of the same solution were given to four rats of weights varying from 287 to 460 g (109-174 µg /kg.) The experiments were terminated after 20, 32, 45 and 60 minutes

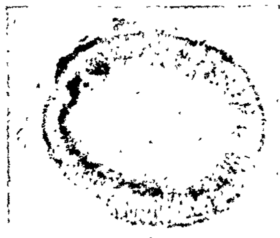


Fig 33. The filling of adrenal cortical vessels shown by empty (× 12)

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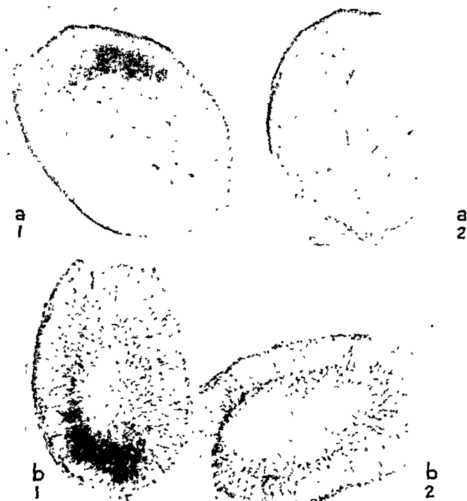


FIG. 32. Microradiographs of the adrenals of rats 30 min. (a_1 and a_2, the upper two sections) and 60 min. (b_1 and b_2, the lower two sections) following intramuscular injection of 0.2 ml. noradrenaline solution. No appreciable filling is noticeable at 30 min., but 60 min. following injection the capillaries of the cortex are clearly visible (× 12).

0.2 ml. of distilled water (141, 137, and 125 μg./kg. respectively), and the rats killed after 20, 40 and 60 minutes when the adrenals were removed. Filling of adrenal cortical vessels was only observed after 60 minutes (Fig. 33) and not after 20 or 40 minutes.

CHAPTER VI

THE INFLUENCE OF THE PITUITARY AND ADRENAL CORTICAL SECRETION ON ADRENAL CORTICAL BLOOD SUPPLY

THE influence of the pituitary on adrenal cortical activity may best be determined in hypophysectomized animals or animals injected with adrenocorticotrophic hormone (ACTH). Since the hypothalamus may also be implicated in any such hypophyseal mechanism (see Long 1952; Groot and Harris, 1952; Harris, 1955), the first experiments on the rabbit (Harrison, 1957) included observations on the effect of adrenaline in three decerebrate and hypophysectomized animals (see p. 7 for technique). One hour after the *intramuscular injection of 1 ml. 1/1000 adrenaline tartrate in all three animals* (455-556 µg./kg.) there was pronounced filling of adrenal cortical vessels.

The effect of adrenaline on the hypophysectomized rat was also observed. Thus two rats hypophysectomized 2 days previously were injected intramuscularly with 0.15 ml 1/1000 adrenaline tartrate (562 and 620 µg./kg. respectively) and were found to have marked filling of adrenal cortical vessels 43 and 45 minutes later (Fig. 34). Another rat, hypophysectomized 5 days previously, was injected intramuscularly with 0.2 ml. 1/1000 adrenaline tartrate, and found to have pronounced filling of adrenal cortical vessels 60 minutes later (Fig. 35). In two rats only the anterior lobe of the pituitary was removed 22 days previously (histological observation of the pituitary remnant

rats 'sham' hypophysectomized (the operation being performed in all particulars except for removal of the pituitary) 11 and 22 days previously also showed filling of adrenal cortical vessels 60 minutes after intramuscular injection of 0 2 ml. 1/1000 adrenaline tartrate solution. From these experiments it may be concluded that the effect of adrenaline on adrenal cortical *blood supply is independent of the pituitary. This does not exclude, however, a* direct effect of ACTH on adrenal cortical vascularization, independent of adrenaline.

Accordingly, in one 2.4 kg. rabbit 10 i.u. ACTH (Organon Laboratories Ltd.) were injected intravenously, followed 37 minutes later by another 10 i.u., and then 40 i.u after a further 43 minutes. No filling of cortical vessels was observable at any time up to 45 minutes after the last intravenous injection,

These experiments therefore demonstrate that other vasoconstrictor substances can influence the intracortical vascularization of the adrenal gland. Noradrenaline is not significantly more effective than adrenaline in causing filling of cortical vessels after intramuscular or intra-arterial injection, however, although it is a more effective vasoconstrictor of arteries other than the arteriae medullae (von Euler, 1956). Adrenochrome, on the other hand, which may be the substance which is finally effective within the gland in producing vasoconstriction of arteriae medullae, is more effective than both adrenaline and noradrenaline when injected intramuscularly or intraperitoneally, causing filling of cortical capillaries after 30 minutes. Further, it appeared evident at this juncture that this mechanism of filling of intracortical vessels in the rat is specific to adrenaline, noradrenaline and adrenochrome, natural products of the adrenal gland, since it did not occur with other vasoconstricting substances such as serotonin, or vasodilators such as histamine or ox kallidin until much later — i.e. 60 minutes after injection. The only exceptions to this rule were in the experiment on the monkey in which histamine injection was complicated by prior injections of adrenaline, and in the rabbit, where histamine acts more rapidly than adrenaline.

At this stage of the researches, therefore, it was considered that a specific response of the adrenal cortical blood supply to the effect of adrenaline, noradrenaline and adrenochrome acting by way of a vasoconstrictor action on the arteriae medullae, had been revealed. Those experiments in which injections had been made but no response obtained were considered as controls The next phase of the experimental project was to determine the part played by the pituitary gland, and of hormones secreted by the adrenal cortex itself, on the vascularization of the adrenal cortex.

(iii) Intra-arterial injection of 5 i.u. ACTH by means of a carotid cannula in four rats weighing 330-385 g. again produced filling of cortical vessels after 40 minutes, but not after 10, 20 or 30 minutes.

In the rat, therefore, injection of ACTH by three different routes does cause filling of adrenal cortical vessels, but only after 40 minutes, even when administered intra-arterially, compared with the 30 minutes required by intra-



FIG 36 Filling of adrenal cortical vessels in a rat subjected to removal of the anterior lobe of the pituitary 22 days previously, as seen by microradiography 60 min after

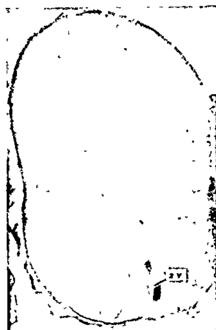


FIG 37 Lack of filling of cortical vessels in a 2 4 kg female rabbit, after intravenous injection of a total of 60 i u ACTH as shown in a microradiograph of a frozen section of the left adrenal removed 40 min. after the last injection of 40 i u ACTH and 2 hr 5 min after the first injection of 10 i u ACTH The adrenal vein (a v) is conspicuous (× 10)

arterial injection of adrenaline and noradrenaline. This would appear to indicate first that there are two independent mechanisms effecting alterations in adrenal cortical blood supply — adrenaline acting directly on the adrenal, and ACTH (whose secretion may, however, be stimulated by the action of adrenaline, although the results of the experiments on hypophysectomized rats indicate that the influence of adrenaline on the vascularization of the

i.e. 2 hours 5 minutes after the first injection, nor any difference in the microradiographic appearance of the adrenal from that seen in normal controls (Fig. 37).

It was decided to repeat these experiments in rats, and three sets of observations were made:



FIG 34 Marked filling of adrenal cortical vessels obtained in a rat hypophysectomized 2 days previously, and injected with 0 15 ml 1/1000 adrenaline tartrate intramuscularly 43 min before the adrenal was removed (× 15)



FIG 35 Microradiographic appearance of a section of an adrenal from a rat which was hypophysectomized 5 days previously, obtained 60 min after intramuscular injection of 0 2 ml 1/1000 adrenaline tartrate All intraglandular vessels are well filled (× 20)

(i) Three rats, 275, 400 and 465 g. in weight, each received an intramuscular injection of 3 i.u ACTH in 0.5 ml sterile distilled water. Forty-one and 60 minutes later there is pronounced filling of adrenal cortical capillaries (Fig. 38).

(ii) Four rats, weighing from 370-415 g., were injected subcutaneously with 5 i.u ACTH in 0 25 ml. sterile distilled water. Forty minutes later filling of intracortical vessels was observed, but not 10, 20 or 30 minutes after ACTH injection.

output therefrom, any effect on adrenal cortical blood supply is secondary and is augmented in order to remove the hormones secreted.

Further evidence of the action of the pituitary in altering adrenal cortical blood supply was obtained in experiments utilizing cortisone acetate. Injections of this compound depress the secretion of ACTH (see Sayers and Sayers, 1948; Sayers, 1950) and might therefore be expected to influence adrenal cortical secretion and blood supply. Accordingly, six rats, weighing 290-415 g., were each injected intraperitoneally with 2.5 mg. cortisone acetate dissolved in 0.5 ml. sterile distilled water. One hour later each rat was anaesthetized, subjected to the standard adrenaline procedure — i.e. intra-arterial injection of Thorotrast together with intramuscular injection of 0.2 ml. 1/1000 adrenaline tartrate (482-690 μg./kg.) – and killed after 55-60 minutes. In all six rats the intracortical capillaries of the gland were well injected, except for those of the outer zona fasciculata, which stands out clearly as a paler, more poorly injected, zone (Fig. 39). In a seventh rat, in which only 2.25 mg. cortisone acetate was administered intraperitoneally 1 hour before intramuscular injection of adrenaline, there was filling of cortical capillaries in all zones, thus suggesting that this dosage is fairly critical. It is interesting to examine the mechanism whereby lack of endogenous ACTH secretion from the rat's own pituitary, effected by depression of the pituitary caused by cortisone, should result in lack of filling of the vessels of only the outer zona fasciculata, whereas those in the remainder of the cortex are well filled. According to Bennett and Kilham (1940), the cortical capillaries can be narrowed by the enlargement of the cells of the outer zona fasciculata

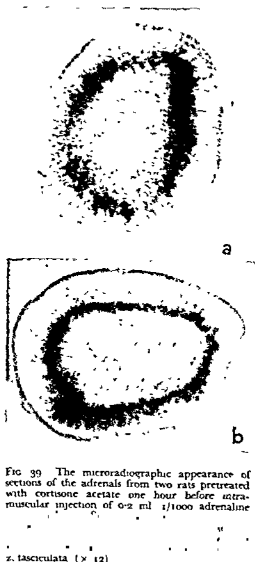


FIG 39 The microradiographic appearance of sections of the adrenals from two rats pretreated with cortisone acetate one hour before intramuscular injection of 0·2 ml 1/1000 adrenaline

z. fasciculata (× 12)

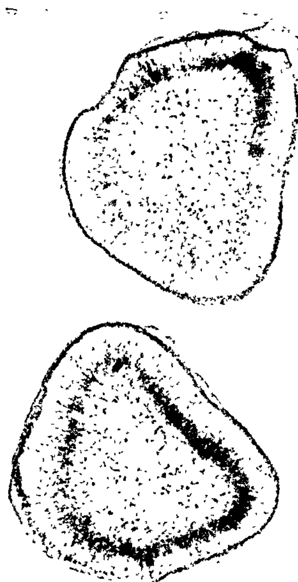


FIG. 38 Two sections from the adrenals of a rat 60 min after intramuscular injection of 3 i.u. ACTH, as seen by *microradiography. The intracortical vessels, except for those in the z. fasciculata in this particular rat, have* been well injected (× 14)

adrenal cortex is unaffected by this procedure) – and, second, that the manner of action of ACTH on adrenal cortical blood supply differs from that of adrenaline. Since ACTH takes longer to exert its effect, and is known to increase cellular activity in the adrenal cortex leading to an increased hormone

and this could be accomplished by injection of ACTH, as already noted, or by increased endogenous ACTH secretion. Such a state is achieved in the remaining adrenal cortex following unilateral adrenalectomy, since the gland is hypertrophied and subjected to a relative increase in ACTH stimulus. Accordingly, in four rats varying in weight from 260-335 g., the left adrenal was removed, and 23 days later the standard adrenaline experiment (i.e. intramuscular injection of 0.2 ml. adrenaline (597-735 µg./kg.) and intra-arterial injection of Thorotrast) carried out; the experiments were terminated after 10, 25, 30 and 40 minutes. There was marked filling of cortical vessels in the remaining hypertrophied adrenals in all cases, even 10 minutes after the adrenaline injection (Fig. 40). Forty minutes after adrenaline injection the microradiographic picture suggests that many haemorrhages have occurred in the cortex.



FIG 41. Microradiograph of sections of a monkey adrenal removed after injections of ACTH and adrenaline. Only vessels in the outer z. fasciculata are filled (× 5)

The remaining adrenal in a unilaterally adrenalectomized rat is therefore much more sensitive to intramuscular adrenaline injection than adrenals in a normal rat. It can only be presumed that such increased responsiveness is caused by a relatively increased endogenous ACTH stimulus, and that an increase in ACTH secretion from the rat's own pituitary is far more effective than injections of ACTH extracted from the pituitaries of other animals. The ACTH used in the experiments described on pp. 41-43 was extracted from pigs' pituitaries, and this may well cause antihormone production in the rat which would render it less efficient than the rat's own endogenous ACTH. In the one rat in which haemorrhages occurred into the adrenal cortex the remaining adrenal was more markedly hypertrophied, as judged by weight increase (19.2 mg. increase as against 2.1-7.1 mg. in the other three rats); in this case it can only be assumed that the increased blood supply was so marked that the walls of the capillary sinusoids of the cortex were unable to withstand the marked hyperaemia and therefore burst.

The effect of ACTH and of cortisone acetate on the vascularization of the adrenal cortex was also determined in two female macaque monkeys. In one monkey weighing 5.02 kg. the intramuscular injection of 40 i.u. ACTH in 1.5 ml. water produced radiographic evidence of only very slight filling of adrenal cortical vessels 38 minutes later which diminished after 10 minutes, at which time 0.12 ml. 1/1000 adrenaline tartrate was injected intramuscularly;

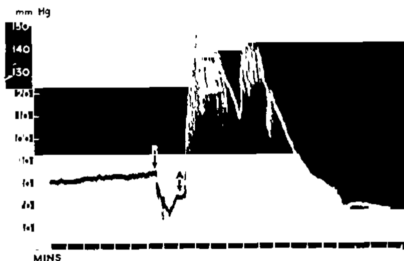
with contained lipoid, and this would accumulate in cortical cells of particularly this region of the cortex in rats subjected to diminished, endogenous ACTH secretion. The fact that the remainder of the cortex is well vascularized,



FIG 40 Filling of adrenal cortical vessels 10 min after intramuscular injection of 0 2 ml 1/1000 adrenaline tartrate shown by microradiography in 3 sections from an adrenal of a rat in which the contralateral adrenal had been removed 23 days previously The sections overlap slightly (× 9)

again indicates an inherent response of the cortical vascularization to adrenaline independent of ACTH secretion.

Release of hormones derived from intracortical lipoid into the blood stream would cause a decrease in cell size, so allowing an increase in capillary volume,



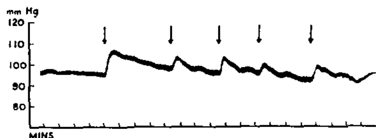


FIG 45 Arterial blood pressure response to five intravenous injections of 0·01 ml 1/1000 adrenaline tartrate solution (at arrows in the figure) in a female macaque monkey pretreated with 40 mg cortisone acetate The rises in blood pressure are transient and not very large



FIG 42. Lack of filling of intracortical vessels in a macaque monkey pretreated with cortisone acetate and injected with adrenaline and histamine Only a few medullary vessels are visible (compare with Fig 30) (× 12)

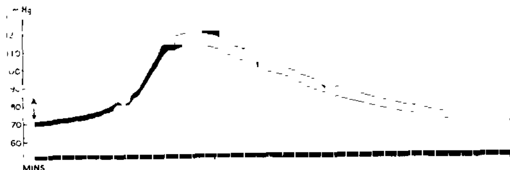


FIG. 43 Arterial blood pressure response to the intramuscular injection of 3 ml 1/1000 adrenaline tartrate solution (injected at A in figure) in a female macaque monkey

ization of blood pressure, and be of value in diminishing the fall of blood pressure in shock, for example.

The monkey experiments further confirm the influence of ACTH and cortisone on adrenal cortical vascularization already observed in the rat. Independent experiments (Grant, Forrest and Symington, 1957) have shown that ACTH increases the flow of blood from the adrenal vein in man. Hicks and West (1958) have also shown that corticosterone exerts a functional control over the tissue levels of both histamine and serotonin in the rat, and this mechanism may be complementary to the control of adrenal cortical vascularization by adrenal cortical hormones acting on ACTH secretion.

this caused a reappearance of the radiographic evidence of minor filling which persisted for another 10 minutes when a further 0.07 ml. 1/1000 adrenaline tartrate caused slight enhancement of the radiographic appearance. Removal of the adrenals and *microradiography of sections of them at this* stage showed filling of vessels in only the outer part of the zona fasciculata of the cortex (Fig. 41). The value of this experiment is in demonstrating a synergism between ACTH and adrenaline injections, and the fact that ACTH exerts its effect mainly, if not entirely, on the outer zona fasciculata.

In the second monkey weighing 4.15 *kg.*, 40 mg. cortisone acetate in 2 ml. aqueous suspension was injected intramuscularly, followed 87 minutes *later by an intramuscular injection of* 3 *ml.* 1/1000 adrenaline tartrate and

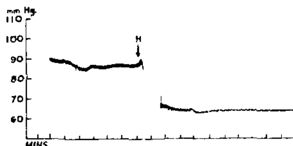


Fig 46 The fall in blood pressure occasioned by the intravenous injection of 2 mg histamine acid phosphate in 2 ml *of solution (at H in the figure) in the same monkey as that* whose blood pressure record is shown in Fig 45 The fall is not as marked as that shown in Fig 31

after a further 1 *hour five injections of* 0 01 *ml* 1/1000 adrenaline tartrate solution injected intravenously over the course of 8 minutes In 11 minutes 2 ml. histamine acid phosphate were injected intravenously. During the whole of this experimental procedure, and over the course of the next 23 minutes, *after which time the monkey was killed with an overdose of nembutal*, there was no radiographic evidence of adrenal cortical filling, and subsequent microradiography of the adrenal sections showed no filling of intracortical vessels (Fig. 42).

Interestingly the blood pressure reaction to the second adrenaline injection (but not to the first) was very much diminished in this animal as compared to that in other monkeys injected with adrenaline or to that in the monkey injected with ACTH (Figs. 43, 44 and 45), as was also the blood pressure fall after histamine injection as compared with that previously reported (p. 36; Figs. 31 and 46). Thus cortisone may well have an influence on the stabil-

in the outer zona fasciculata and zona glomerulosa, which are not as well filled as in the experiments in which adrenaline was injected intramuscularly (Figs. 25 and 26). Similarly in three rats weighing 347, 370 and 382 g. in which 0.2 ml. normal saline was injected intraperitoneally, there is some filling of adrenal cortical vessels 20, 30 and 45 minutes after injection.

It was obvious, therefore, that the injection of normal saline intramuscularly and intraperitoneally in the rat is far from being innocuous, and that such experiments could not be considered as absolute controls, since a positive response was obtained in some of the animals. Nevertheless, in others there was no filling of cortical vessels, and therefore the experiments involving injection of normal saline at later time periods clearly had to be considered as evidence of a positive reaction to this experimental procedure. This is not as surprising as may appear at first sight, however, since the injection of 0.2 ml. saline, or of any other fluid, into a rat weighing 300 g. is comparable to an intramuscular injection of some 50 ml. into an average man, a procedure which would not be unaccompanied by a certain amount of stress. Saline injection, it was presumed, therefore, has an effect on adrenal cortical vascularization by virtue of its production of a stress situation, and further experiments were clearly necessary to determine whether filling of cortical vessels could be obtained in a more basic experiment in which Thorotrast was injected intra-arterially in the rat anaesthetized with ether, without the injection of any other medium by any route. Accordingly, in ten rats, varying in weight from 276-380 g. and anaesthetized with ether, the microradiographic appearance of the adrenal was determined 10, 20, 30, 40, 50, 60 and 70 minutes after intra-arterial injection of Thorotrast; no filling of adrenal cortical vessels was observed, except in two rats at 50 and 70 minutes in which there was very slight filling in the zona reticularis or outer zona fasciculata (Fig. 27), despite the fact that ether anaesthesia alone is considered as an agent producing a rise in the blood level of ACTH, even in adrenalectomized rats (Hodges and Vernikos, 1959). Such an experiment was therefore decided as the basic control from which all other experiments could be interpreted particularly since the acute release of ACTH in response to the stress of ether anaesthesia is actually *diminished* or even absent after injections of adrenaline (Kitay, Holub and Jailer, 1959). Eight further basic experiments of this nature demonstrated that the amount of Thorotrast injected intra-arterially, in the range 3-5 ml., did not affect the response in rats varying in weight from 292-406 g.

It remained to determine the manner of action of saline in effecting a response in adrenal cortical vascularization. Since this may be mediated by a reflex mechanism exerted through the splanchnic nerves, the influence of Dibenamine on the saline response was examined. Five mg. Dibenamine

CHAPTER VII

CONTROL EXPERIMENTS: THE INFLUENCE OF THE INJECTION OF NORMAL SALINE AND NERVOUS STIMULI

ALTHOUGH the experiments in which various media were injected and no response in adrenal cortical vascularization obtained were considered as controls in the earlier work, it was nevertheless thought desirable to undertake experiments in the rat to determine the effect of injections of normal saline on adrenal cortical vessels.



FIG 47 (a) Microradiograph of a section from an adrenal of a rat 60 min after intramuscular injection of 0 2 ml normal saline The intracortical capillaries, except those in the outer z fasciculata and z glomerulosa, are well filled (× 13)



(b) Microradiograph of an adrenal section from a rat treated similarly to that shown in (a), 70 min after injection of 0 2 ml normal saline intramuscularly (× 12)

Six rats varying in weight from 275-320 g. were each injected intramuscularly with 0.2 ml. normal saline into the thigh muscles of the hind leg, as in the adrenaline experiments. At 20 and 30 minutes after injection the ... evidence of only very slight filling ... injection, however, ... (Fig. 47) except

No filling of adrenal cortical sinusoids was obvious in the remaining adrenal 9, 10 or 20 minutes after injection; after 30 minutes, however, filling of intra-cortical vessels was seen. If unilateral adrenalectomy had sensitized the response of the remaining adrenal to saline injection, it might be thought that a response would have been obtained much earlier than this, because as described on p. 46, filling of adrenal cortical vessels in a hypertrophied adrenal after contralateral adrenalectomy occurred 10 minutes after adrenaline injection. However, the maximum response in cortical vascularization was obtained in the rat in which the greatest increase in adrenal weight occurred (19 mg increase as against 5-8 9 mg.). This suggests that increased endogenous ACTH activity in unilaterally adrenalectomized rats does not expedite, although enhancing, the response of adrenal cortical vessels to normal saline injections, which are therefore likely to exert their effect by some mechanism independent of the pituitary.

In further experiments the influence of a locally applied nervous stimulus was therefore determined. At first crude stimuli were employed: a hypodermic needle was inserted into the thigh muscles of a rat, moved about therein for 5 seconds, and then withdrawn. In all cases, in eight rats varying in weight from 235-405 g. there was marked filling of adrenal cortical sinusoids, particularly in the zona glomerulosa in some rats, at intervals from 15 to 60 minutes later, with haemorrhages into the zona fasciculata (Figs. 48 and 49). In another rat weighing 290 g. the right femoral nerve was exposed and crushed with artery forceps. Thirty minutes later marked haemorrhages and filling of adrenal cortical vessels were evident (Fig. 50). In two further rats the right femoral nerve was exposed and stimulated electrically for 5 seconds at 5 volts and 2.5 amp.



Fig 49 Filling of adrenal cortical capil-

occurred in the z fasciculata (× 12)

hydrochloride (Givaudan Delawanna Inc.) in 0.2 ml. solution was injected intravenously into a jugular vein 1 hour before intraperitoneal injection of 0.2 ml. normal saline in three rats weighing 324, 332 and 334 g. In all three rats microradiography of the adrenals removed 1 hour after the saline injection revealed filling of vessels in all zones except the outer zona fasciculata. Two of the rats showed haemorrhages into the zona fasciculata. Dibenamine did not prevent this response, therefore. Since Dibenamine is claimed to block the effect of adrenaline itself (Nickerson and Nomaguschi, 1953) but not the passage of impulses across the post-ganglionic synapse in some adrenergic nerves, the effect of a non-specific stressor may be exerted on adrenal cortical vascularization through the intermediary of the sympathetic nervous system. Dibenamine, however, did not even prevent the adrenal cortical vascular response to adrenaline injection. In three rats weighing 275, 328 and 352 g., 0.1 ml. (containing 5 mg.) of a solution of Dibenamine hydrochloride containing 500 mg. in 10 ml., was mixed with 0.1 ml. distilled water and injected into a jugular vein of each rat 1 hour before intraperitoneal injection of 0.2 ml. 1/1000 adrenaline tartrate solution. One hour later in all three rats there was filling of adrenal cortical vessels in all zones. In attempting to understand this apparent failure of Dibenamine to block the action of adrenaline, it should be noted that Paschkis *et al.* (1949a and b) found Dibenamine to act as an 'alarming stimulus', and so, whilst blocking the direct effect of adrenaline on the adrenal cortical vessels, it may itself act on the cortex to produce vascular filling. The action of saline on the cortical vessels would therefore be augmented by Dibenamine, and this would explain the cortical haemorrhages occurring in two such rats.



FIG 48 Filling of adrenal cortical sinusoids 15 min after insertion of a hypodermic needle into the thigh muscles of a rat The outer $\frac{1}{10}$ – $\frac{1}{5}$ of the cortex is not very well injected (× 13)

An experiment with unilaterally adrenalectomized rats was carried out to determine the reaction of the remaining hypertrophied gland to injections of normal saline. The left adrenal of five rats, varying in weight from 317-410 g was removed and 18 days later 0.2 ml. normal saline injected intramuscularly.

No filling of adrenal cortical sinusoids was obvious in the remaining adrenal 9, 10 or 20 minutes after injection; after 30 minutes, however, filling of intracortical vessels was seen. If unilateral adrenalectomy had sensitized the response of the remaining adrenal to saline injection, it might be thought that a response would have been obtained much earlier than this, because as described on p. 46, filling of adrenal cortical vessels in a hypertrophied adrenal after contralateral adrenalectomy occurred 10 minutes after adrenaline injection. However, the maximum response in cortical vascularization was obtained in the rat in which the greatest increase in adrenal weight occurred (19 mg. increase as against 5-8.9 mg.). This suggests that increased endogenous ACTH activity in unilaterally adrenalectomized rats does not expedite, although enhancing, the response of adrenal cortical vessels to normal saline injections, which are therefore likely to exert their effect by some mechanism independent of the pituitary.



Fig 49 Filling of adrenal cortical capil-

In further experiments the influence of a locally applied nervous stimulus was therefore determined. At first crude stimuli were employed: a hypodermic needle was inserted into the thigh muscles of a rat, moved about therein for 5 seconds, and then withdrawn. In all cases, in eight rats varying in weight from 235-405 g. there was marked filling of adrenal cortical sinusoids, particularly in the zona glomerulosa in some rats, at intervals from 15 to 60 minutes later, with haemorrhages into the zona fasciculata (Figs. 48 and 49). In another rat weighing 290 g. the right femoral nerve was exposed and crushed with artery forceps. Thirty minutes later marked haemorrhages and filling of adrenal cortical vessels were evident (Fig. 50). In two further rats the right femoral nerve was exposed and stimulated electrically for 5 seconds at 5 volts and 2.5 amp.

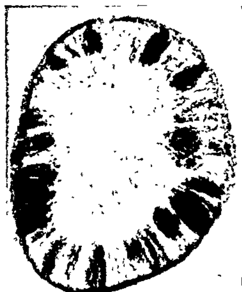


FIG 50 Haemorrhages, and filling of adrenal cortical sinusoids, in a rat 30 min after crushing the right femoral nerve The capillaries of the z glomerulosa have been injected (× 12)

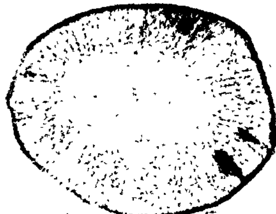


FIG 51 Filling of intracortical vessels in a rat 30 min after stimulating the right femoral nerve (× 12)



FIG 52 Microradiograph of a sect on from

at a frequency of 10 stimuli per second. Thirty minutes later there was marked filling of adrenal cortical vessels particularly of the zona glomerulosa, with haemorrhages into the zona fasciculata (Figs. 51 and 52). Such experiments suggest that a nervous stimulus applied to the hind limb of a rat can effect filling of adrenal cortical vessels, presumably by reflex activity. It was therefore deemed advisable to subject rats to other forms of stress and observe their effect on adrenal cortical blood supply.

CHAPTER VIII

THE EFFECT OF STRESS ON ADRENAL CORTICAL VASCULARIZATION

Two methods of acute stress were utilized — the exposure of the whole rat to heat and to cold. Eighteen rats varying in weight from 272-447 g. were utilized for this series of experiments. Five ml. Thorotrast was injected into the left carotid artery of all the animals, using ether anaesthesia. Following this injection the rats were allowed to recover consciousness. Five of the rats were then subjected to heat shock in a hot room for 1 hour at 110° F Dry Bulb

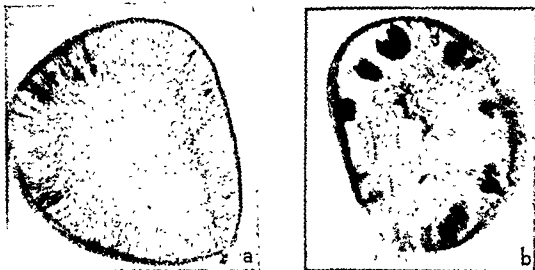


FIG. 53 Microradiographs of sections of rat adrenals following subjection to high (a) and low (b) temperatures. The adrenal cortical capillaries, including those in the z. glomerulosa, are well filled. In the rat subjected to cold, haemorrhages have occurred into the z. fasciculata (× 12)

temperature and 83° F Assman Wet Bulb temperature. Eight rats were placed in a refrigerator at -4° C (39° F) for 1 hour. The remainder of the rats served as controls and were left in a room at a temperature of 20° C (68° F). All of the animals subjected to high or low temperature showed marked filling of adrenal cortical sinusoids (Fig. 53): in one rat subjected to heat, and one to cold, haemorrhages occurred into the zona fasciculata of the cortex. The zona glomerulosa was particularly well filled in all the rats. In the control

animals, however, there was no filling of adrenal cortical vessels or only a very slight filling in some parts of the zona fasciculata.

For investigation of the effect of long-continued stress on adrenal cortical vascularization, seventeen rats varying in weight from 302-491 g. were used. These rats had been implanted with a 20 mg. benzpyrene pellet into one or other or both thighs 9 months previously. In some rats the femoral and pudo-



FIG. 54 Patchy filling of adrenal cortical vessels in a rat developing a tumour from a benzpyrene implant as shown in a section of the adrenal subjected to microradiography following intra-arterial injection of Micropaque Haemorrhages are present (× 10)



FIG 55 More extensive filling of adrenal cor-

epigastric vessels had been ligated in order to determine the effect of ischaemia on the growth of the experimental tumour, in an *investigation conducted by* Dr. J. L. Braithwaite of this Department, in collaboration with Professor Haddow, Chester Beatty Research Institute, London. Thirteen of the rats developed tumours, and it was therefore possible to determine the effect of the chronic stress created by such a tumour on adrenal cortical vascularization. In all rats which did develop tumours, there was some patchy filling of adrenal

cortical vessels in some areas of the cortex (Fig. 54), and in one rat filling of the central vein (Fig. 55) when Micropaque was injected at the time of sacrifice, using the method of incision of the plantar surface of the hind feet in order to prevent injection at too great a pressure. These appearances do not differ significantly from that in some controls (see Fig. 27e). In



Fig 56 The microradiographic appearance of an adrenal section from a rat not developing a tumour from a benzpyrene implant Only arteriae medullae are visible (× 17)

the rats not developing tumours, however, only arteriae medullae (Fig. 56) were visible on microradiography.

It may be concluded therefore, that acute stress has a profound effect on adrenal cortical vascularization, causing filling of cortical sinusoids. Since this filling occurs throughout the cortex, in contrast to the lack of filling of the outer zona fasciculata following saline injections, the possibility that the latter may have some other mechanism of action, such as the liberation of tissue metabolites, must be considered.

CHAPTER IX

SYNTHESIS

It is extremely difficult to inject the adrenal cortical sinusoids of the normal animal which is at rest and not subjected to environmental or functional modification, even if the animal is anaesthetized with ether (pp. 33, 53). From the illustrations published by other authors (for example, Gersh and Grollman, 1941) it is obvious that similar difficulties have been experienced in obtaining a complete injection of the capillary sinusoids of the adrenal cortex of experimental animals. If the degree of vascularization of an organ may be taken as reflecting its functional activity, the observations reported in the preceding chapters indicate that various stimuli effect an overall increase in adrenal cortical vascularization and thereby increased cortical activity. There appear to be three mechanisms responsible for these alterations:

(1) Vasoconstriction of arteriae medullae, effected by humoral agents, the most specific of which appear to be adrenaline, noradrenaline and adrenochrome.

(2) The influence of ACTH. This is almost certainly exerted directly on the adrenal cortical cell, and any alterations in cortical vascularization caused by it are therefore probably secondary to the cellular changes

(3) Vasoconstriction of arteriae medullae effected by the sympathetic nervous system, probably brought into being by reflex activity, and mediated finally through the splanchnic nerves.

Since injection of normal saline into a rat acts as an efficient stressor, presumably acting via mechanism (3), or by the liberation of a tissue metabolite at the site of injection, it is important to analyse the proportionate influence exerted by this factor when adrenaline is injected Saline injections are more rapid in their effect than injections of adrenaline, which is as would be expected if their effect is mediated by means of a reflex action. The relative delay in response of adrenal cortical blood supply to the injection of adrenaline may be explained either on the grounds of the vasoconstrictor effect of this substance (*a*) at the site of injection, thus delaying its entry into the general circulation, and (*b*) on the arteries supplying the adrenals and so prolonging the time interval between injection and the vasoconstriction of arteriae medullae, or, if adrenaline exerts its effect partially or wholly through a reflex mechanism, by the influence of adrenaline on the transmission of impulses in sympathetic ganglia (Bulbring and Burn, 1942)

This work has demonstrated the possibility that adrenaline, or some similar compound secreted by the adrenal medulla, may have a *specific* and *direct* influence on the arteriae medullae, so augmenting adrenal cortical vascularization. Certain observations suggest this possibility:

A The medulla of the adrenal gland exerts a control, under numerous circumstances, directly on the adrenal cortex, since the effect of adrenaline in causing a lymphopenia is not evident in the bilaterally adrenalectomized dog (Malméjac and Gross, 1954).

B. Malméjac *et al.* (1954) eliminated the influence of reflex activity of the nervous system in examining the effect of adrenaline injections by perfusing the left adrenal of a dog B with blood from another dog P which was injected with adrenaline. Physiological doses of adrenaline of the order of 0 2-1 µg./kg./min. produced slight hypertension in dog P, whether adrenalectomized or not, and 30-40% augmentation in outflow of venous blood from the left adrenal of dog B. If a dose of 2-4 µg /kg./min. of adrenaline was injected into dog P, it showed marked hypertension which corresponded to an augmentation in adrenal venous outflow of dog B, and this was followed by a diminution in the outflow, falling to a level 40-50% below normal The injection of a dose of 0.25 mg./kg./min., or 0 5-1 mg. in a single injection, in dog P, produced complex responses There was first a pronounced hypertension in dog P corresponding to an augmentation of adrenal venous outflow of dog B; the latter then diminished rapidly as dog P's arterial pressure started to fall, until finally it fell to one-third or one-quarter of its initial value.

C By [illegible] of the adrenal of a dog with blood from a se[illegible] M[illegible] 1955, have shown that small and large doses of [illegible] P diminished the secretion of adrenaline by the left adrenal of dog B. This could be explained by the vasoconstriction of arteriae medullae in the left adrenal of dog B. The effect was independent of the nerve supply of the adrenal (Malméjac *et al.*, 1955b).

D. If adrenaline acts as a specific humoral transmitter in the hypophyseal portal circulation for the discharge of ACTH from the anterior lobe of the pituitary, it would not necessarily be expected that noradrenaline and adrenochrome are also effective in altering adrenal cortical vascularization, although other vasoconstrictors (e g. serotonin) are not as effective.

E The extent of filling of cortical capillaries in the experiments using cortisone acetate (p. 45) and saline injections (p 52) differs from that following adrenaline injections. Adrenaline causes a filling of capillaries throughout the cortex, particularly in the zona glomerulosa (Figs. 25 and 26), and including all the zona fasciculata. Following treatment with cortisone acetate or

saline injections, however, a zone involving the outer zona fasciculata is not filled (Figs. 39 and 47).

F. The effect of adrenaline on cortical vascularization is independent of the pituitary in both the rabbit and the rat, as is also the effect of adrenaline in increasing output of adrenal cortical hormones in the rat (Vogt, 1944). Speirs and Meyer (1949) have also reported that adrenaline causes eosinopenia in hypophysectomized mice, although stress and adrenaline injections do not cause eosinopenia in adrenalectomized mice and rats.

The vascularization of the adrenal cortex, nevertheless, is responsive to activity of the anterior lobe of the pituitary, as shown by the experiments utilizing ACTH injections (in the rat), prior injection of cortisone acetate (rat and monkey), and unilateral adrenalectomy (rat), and, as the experiments on unilaterally adrenalectomized rats demonstrate, the response to the rat's own endogenous ACTH is more dramatic than that utilizing commercial preparations of ACTH extracted from the pituitaries of other animals.

It has been claimed that increased output of cortical hormones in the perfused gland following administration of ACTH is dependent upon blood flow through the cortex (Hechter *et al.*, 1951). Whether the increase in hormone output is due to stimulation of the cortical cells and synthesis of hormone by the increased blood flow, or to the removal of greater quantities of preformed hormones, is uncertain; Hechter *et al* claim that the former occurs. *In vivo* it is difficult to envisage how ACTH could produce an alteration in the degree of vascularization of the adrenal cortex, other than as a response to increased cellular activity within the gland. If this be the case, it would be expected that the changes in adrenal cortical vascularization produced by adrenaline and ACTH should differ, and there is evidence for this. The time relationships of the response in the two cases differ, and there are differences in the zonal response. It is proposed that adrenaline acting directly on the adrenal cortex effects a response in all zones, whereas evidence of ACTH activity, or lack of activity, is visible chiefly in the outer zona fasciculata, in the rat, thus agreeing with previous theories regarding functional correlation with the various zones of the adrenal cortex (see Swann, 1940; Deane and Greep, 1946; Greep and Deane, 1947a and b; Cain and Harrison, 1950). This is seen very clearly following treatment with cortisone acetate, and is probably a reflection of the degree of compression of cortical capillaries governed by the content of lipoid in adjoining zona fasciculata cells. This may explain why filling of capillaries throughout the cortex is not observable until 40 minutes after an ACTH injection in the rat, not at all in the rabbit, and only slightly in the monkey experiment, where the filling was restricted to the outer zona fasciculata (although in this particular observation the experiment was also complicated

by adrenaline injections). Only when the size of cells in the zona fasciculata is diminished by loss of lipoid from them, under the influence of ACTH, does the capillary bed throughout all the zona fasciculata and zona reticularis become patent.

Long (1947a, 1952) has postulated the existence of at least two mechanisms which control adrenal cortical activity, one a self-regulating humoral system, possibly involving the relative blood concentrations of ACTH and adrenal cortical hormones, the other representing the response of the sympathetic nervous system. He presumes that they are both effected by variations in the level of ACTH secretion. Since he and his collaborators found no significant alteration in ascorbic acid content of the adrenals following adrenaline injection in the hypophysectomized rat, he claims that adrenaline only acts by the intermediary of the anterior pituitary, and although considering the possibility of direct action of adrenaline on the adrenal cortex in 1947 (Long, 1947b), did not do so later (Long, 1952). Yet there are possible objections to the concept of adrenaline causing release of ACTH. Long (1952) himself has recognized that there are certain limitations to the use of the ascorbic acid depletion method for detecting increased adrenal cortical secretion under all circumstances. Ascorbic acid may be practically absent from the gland without noticeably affecting its capacity to secrete, and a normal content of ascorbic acid may be present with unmistakable indications of increased secretory activity.* Further, in the experiments in which Long injected adrenaline into hypophysectomized rats, four injections of 200 μg./kg. were given during a 4-hour experiment and Gershberg, Fry, Brobeck and Long (1950) have shown that while rats exposed to cold show an initial fall in adrenal ascorbic acid, continued exposure is accompanied by a return to normal levels. In addition, the maximum alteration in adrenal cortical vascularization in the rat following adrenaline injections occurs 1 hour after injection, but not 2 and 4 hours following injection. If adrenaline does cause increased ACTH secretion, it may act on hypothalamic centres which may or may not release a humoral transmitter which is conveyed to the anterior pituitary by the hypophyseal portal circulation to release ACTH, or adrenaline itself might act as the humoral transmitter in the hypophyseal portal circulation. If acting on hypothalamic centres, adrenaline may still exert its vasoconstrictor effect on the arteriae medullae by efferent impulses conveyed from the hypothalamic nuclei to the adrenal cortex. It is unlikely that adrenaline itself is the humoral transmitter, since the vessels of the hypophyseal portal system contain smooth muscle in their walls (Stanfield, 1960) and could therefore be constricted by adrenaline or other vasoconstrictors. Finally, Hume and Wittenstein (1950)

* More recentl[illegible]

report that section of the pituitary stalk in dogs with interruption of the hypophyseal portal system does not alter the eosinopenic response to injections of adrenaline. Such an observation could be explained on the grounds of direct action of adrenaline on the adrenal. Hume and Wittenstein also claim that certain hypothalamic lesions abolish the eosinopenic response to adrenaline, and found that high spinal cord section abolishes the response to trauma of the lower limbs. They conclude that the hypophyseal portal circulation is unnecessary, but that afferent impulses reaching hypothalamic centres are essential, for ACTH secretion. The eosinopenic response is an indicator of adrenal cortical secretion, however, and not *a priori* of ACTH activity. These results, in fact, may be taken as evidence for the action of adrenaline and/or the nervous system directly on the adrenal. Further evidence implicating pathways in the central nervous system and the secretion of adrenaline, is provided by Anderson *et al.* (1957) in experiments on dogs in which the midbrain and cervical cord were transected; the reticular formation may also be involved (see Stewart, 1959).

A barrier to the acceptance of a specific role for intrinsic adrenaline activity in causing ACTH liberation is the almost universal failure of attempts with adrenergic blocking agents to interfere with ACTH release. Partial blockades of the mechanism by ergotamine (Ronzoni and Reichlin, 1950) and by Dibenamine (Seifter *et al.*, 1949; Paschkis *et al*, 1950; Ronzoni and Reichlin, 1950) have been reported. Not only was the degree of blockade slight but no attempt has been made to test whether even the partial effect was actually due to adrenergic blocking properties of the drugs; this is particularly relevant since Paschkis *et al.* (1949a and b) found Dibenamine itself to act as an 'alarming stimulus'.

Whether adrenaline exerts its effect directly on the adrenal cortex by constricting arteriae medullae, by acting on hypothalamic centres, or by stimulating ACTH secretion, the danger is to postulate and emphasize one theory to the exclusion of others, whereas in fact such mechanisms may act synergically and there is evidence in the present work (p 47) that ACTH and adrenaline may act in this way. A direct effect of functional alterations on the adrenal cortex, independent of the pituitary, cannot be excluded, and has been overlooked in previous researches. Sayers and Sayers (1948) claim that increased secretion from the adrenal cortex occurs to meet the needs of the peripheral tissue cells for the adrenal cortical steroids, and implicate ACTH activity, although admitting that the prompt response of the adrenal cortex to sudden changes in the external environment is compatible with neural regulation.

The experiments involving nerve stimulation, in this work, suggest that a reflex mechanism may be involved in the constriction of arteriae medullae. The most important observation implicating the sympathetic nervous system

in adrenal cortical activity is provided by Robinson and Munro (1958) who have shown that complete transverse lesions of the spinal cord in human subjects, if located above the level of T5 (i.e. above the level of exit from the spinal cord of sympathetic nerve fibres passing in the splanchnic nerves) produce features indicative of a fall in adrenal cortical secretion, whilst similar lesions below the level of T5 produce no such signs of adrenal cortical hypoactivity. A descending pathway in the spinal cord is therefore suggested. This evidence also supports the conclusion that the effect of such a lesion is not mediated by ACTH.

The alteration in adrenal cortical blood supply produced by injections of normal saline is also presumed to act by a reflex mechanism. It is therefore significant that the sensitization to adrenaline effected by increased ACTH secretion is not observed (p. 55) in rats which are unilaterally adrenalectom- [illegible] This would be expected [illegible] the sympathetic nervous [illegible] drenal cortex rather than indirectly by way of the sympathetic nervous system. Long (1952) has also provided evidence for a reflex mechanism acting on the adrenal cortex. A few drops of 10% saline injected beneath the skin of the scalp of normal rats causes eosinopenia in 1 hour, which is still present 4 hours later. This was not observed, however, in rats in which the spinal cord had been transected at the level of T3

The experiments of Recant, Hume, Forsham and Thorn (1950) in which two completely sympathectomized dogs showed eosinopenia 4 hours after injection of 2 ml of 37% formalin intramuscularly have been quoted (Sayers, 1951) as evidence against the 'direct action of adrenaline' hypothesis, but they do not exclude the liberation of some substance from the tissues at the site of formaldehyde injection, similar to histamine or kallidin, which may then act directly on the adrenal cortex.

Indeed, the mechanisms of action of different forms of stress on the adrenal cortex may not necessarily be identical· the likelihood is that they exert their effect either by a reflex mechanism (as in the injection of saline), or by a humoral mechanism, which may or may not exert its effect by constriction of arteriae medullae, or possibly through the intermediary of the anterior pituitary (as with subjection of the animal to high or low temperature). The extent of filling of adrenal cortical capillaries caused by subjection of the rat to high temperature (Fig. 53) certainly resembles that in rats injected with adrenaline, particularly in the filling of zona glomerulosa capillaries.

The rabbit does not show a positive response of adrenal cortical vascularization to ACTH injections as does the rat (p. 41). In a further experiment it was also demonstrated that the rabbit does not respond to noradrenaline

injections by filling of adrenal cortical capillaries. The intravenous injection of 40 ml. (400 μg.) of a solution of noradrenaline (Levophed) containing 2 mg. in 200 ml. sterile normal saline over a period of 46 minutes, and then after 1 hour's interval the intravenous injection of a further 60 ml. over a period of 14 minutes, in a 2.49 kg. male rabbit, failed to produce radiographic or microradiographic evidence of filling of adrenal cortical vessels different from that in a normal rabbit. The rabbit may therefore react differently from other mammals in its adrenal cortical response, and it is noteworthy that many of the experiments performed by Harris and his colleagues (see Harris, 1955) have been carried out on this animal.

However, since the response of the adrenal cortical circulation in the rabbit differs with adrenaline and noradrenaline injections, and since Elmadjian, Hope and Lamson (1957) have shown that certain kinds of stress, e.g. aggressive emotional displays, are related to increased excretion of noradrenaline, whereas tense, anxious but passive emotional displays are related to increased excretion of adrenaline, the response of the rat adrenal circulation to several kinds of stress, both acute and chronic, was determined in this investigation. It was found that the types of acute stress used cause increased cortical blood supply, and this may be related to the fact that both adrenaline and noradrenaline produce increased cortical blood supply in the rat; or, since it is known that adrenaline is liberated in stress, and the extent of filling of adrenal cortical vessels following the stressors used is similar to that produced by administration of adrenaline alone, the forms of acute stress utilized in this investigation may exert their effect through the liberation of adrenaline

It is of importance that the mechanism of diversion of blood through the vessels of the adrenal cortex should be considered in relation to blood flow through the gland, yet few observations have been made on the experimental modification of adrenal blood flow (see Sapirstein and Goldman, 1959) Biedl (1916) showed that following stimulation of the peripheral stump of the splanchnic nerve there is a marked diminution of blood volume in the adrenal vein Burton-Opitz and Edwards (1917) have shown that the amount of blood passing through the adrenal vein in dogs increases to double its value on stimulation of the splanchnic nerve. Hallion (1921) obtained marked diminution in volume of the adrenal gland on excitation of the peripheral end of the splanchnic nerve Neumann (1912) found that adrenaline injection increases blood flow through the adrenal vein. Investigations of the blood flow through the adrenal vein (reviewed by Heinivaara, 1954) suggest that the adrenal vein itself, by the contraction and relaxation of its muscle wall, can vary blood flow through the vessel. Velican (1948) claims that the cranial part of the right adrenal vein consists of longitudinal fibres which are directly continuous with those of the inferior vena cava whereas the caudal portion of the adrenal vein is composed of muscle fibres

which interlace and cross each other, eventually to surround the aperture at the site of confluence between adrenal vein and inferior vena cava. He claims that this mechanism is concerned with the regulation of blood flow through the gland in man. The ingenious cross-circulation experiments of Denton, Goding and Wright (1959), designed to determine whether the adrenal cortex responds directly to changes of plasma sodium and potassium concentration in the carotid artery of the sheep, indicate that ionic changes in the blood perfusing the adrenal may be a contributory cause of changes of electrolyte-active steroid secretion occurring in sodium deficiency, and that these changes are not due to ACTH. Severe sodium depletion causes a 25-40% reduction of blood flow through the gland.

It is conceivable that the changes in intracortical vascularization observed in these experiments could occur without any pronounced alteration in blood flow, as already noted (Harrison, 1957). The flow into the adrenal vein predominantly through several arteriae medullae could be identical with that occurring solely through cortical capillaries, since the calibre of arteriae medullae is very much greater than that of cortical capillaries. Vogt (1944) found that the increased output of cortical hormones from the left adrenal of eviscerated dogs with the splanchnic nerves cut is independent of both blood pressure and blood flow through the adrenal It is obvious that the relationships between adrenal cortical hormone output, distribution of blood within the gland, and the total blood flow through the adrenal need thorough investigation. It is important to recall that Ackermann and Arons (1958) have shown that blood flow through the thyroid gland can be influenced by administration of adrenaline, though they are doubtful whether this change in flow has any significant effect on the hormone output. It is also of interest that Kuschinsky, Vorherr and Trendelenburg (1957) found that intravenous injections of adrenaline and noradrenaline cause increased filling of renal vessels, and that the adrenals were responsible for this filling, since it did not occur in adrenalectomized animals. Probably insufficient attention has been paid, in the past, to local mechanisms independent of the pituitary proceeding in glands other than the pituitary, and effected by variations in blood flow through, and distribution of blood within, them.

In certain of the rats and one monkey in this investigation haemorrhages occurred into the adrenal cortex. In all cases the adrenals had been subjected to excessive stimulation and, in the experiments on unilaterally adrenalectomized rats, were markedly hypertrophied. Selye and Stone (1950) claim that hyperaemia of the adrenal cortex is a common accompaniment of all types of overstimulation; occasionally, extreme corticotrophic stimulation by anterior pituitary extracts, or exposure to stress, results in almost complete haemorrhagic infarction of the cortex, similar to that seen in Waterhouse-Friderichsen

syndrome in man. The fact that the medulla is unaffected in this syndrome is explained by the presence of the arteriae medullae. The adaptation of the adrenal cortex is dependent upon the severity of the stress and the period of subjection to the stress – i.e. the stage in the adaptation syndrome (Selye, 1950) reached. If the gland is required to adapt itself too quickly, as in the case of sudden and severe stress, the mechanism is strained and haemorrhage may occur into the cortex, as a result of the sudden hyperaemia. The rate of adaptation in individual animals must necessarily differ, the adrenals of some animals being capable of withstanding a greater stress than others, so it is likely that the haemorrhages occurring in the experiments reported here are due to the inability of the individual gland to adapt itself to the experimental conditions without damage.

The investigations described in this work demonstrate that adrenaline and other agents have a profound effect in altering the distribution of blood within the adrenal cortex. A mechanism existing in the gland enables this to be achieved by the proximity of the cortex and medulla of the adrenal gland, since the changes are accomplished by a constriction of arteries supplying the medulla and a diversion of blood into the vessels supplying the cortex. Phylogenetically the cortex and medulla first become associated in the land vertebrates, and it may therefore be presumed that this association is brought about by the increased subjection to stress experienced by terrestrial forms in comparison with the more constant homeostatic environment appreciated by aquatic vertebrates.

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